5. Tissue Optics

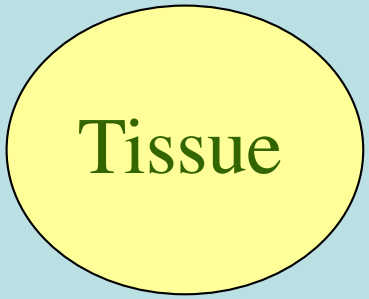
5.1. **Tissue optical parameters**
5.2. The radiative transport equation (RTE)
5.3. Approximations of the RTE
5.4. Measurement methods

What is Tissue Optics ?

Definitions from dictionary

- **Optics**: a science that deals with
  - genesis and propagation of light
  - changes that it undergoes and produces
- **Tissue**: an aggregate of cells
  - usually of a particular kind together with their intercellular substance that form one of the structural materials of a plant or an animal

What is Tissue Optics ?



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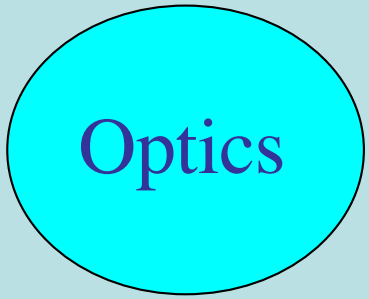


= Tissue Optics

Subject of Interest Investigating Principle

We are interested in …

- Constituents of Tissue
- Propagation of Light
- Interaction between Light and Tissue
- Diagnostic and Therapeutic Implications

Is Tissue Optics Special ?

Many similarities with the propagation of light through the atmosphere !

Three Major Components

- Optical Source – Sun
- Medium - Atmosphere
- Detector – Human Eyes

Interaction between light and particles in atmosphere

- Absorption
- Scattering (Rayleigh & Mie)

Objectives of Tissue Optics

Key Question:
How many photons per second will reach the tissue chromophore and be absorbed?

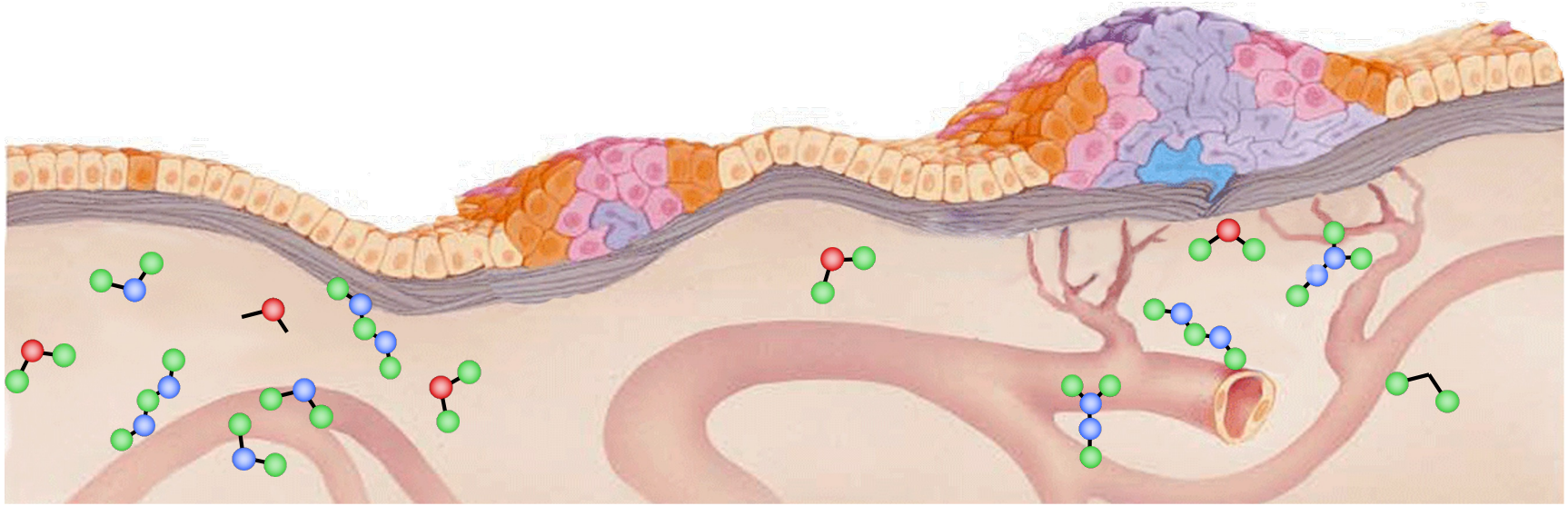
Absorption process is important because it transfers energy to tissue

1. To find the light energy per unit area per unit time that reaches a target chromophore at some position
2. To develop tissue characterization methods based on the absorption and scattering properties

Interest of detecting optical signals from tissues

- Large number of Biological Molecules can be characterized with light.

  → **Functional** and **Structural** information



Ultimate Goal of Tissue Optics

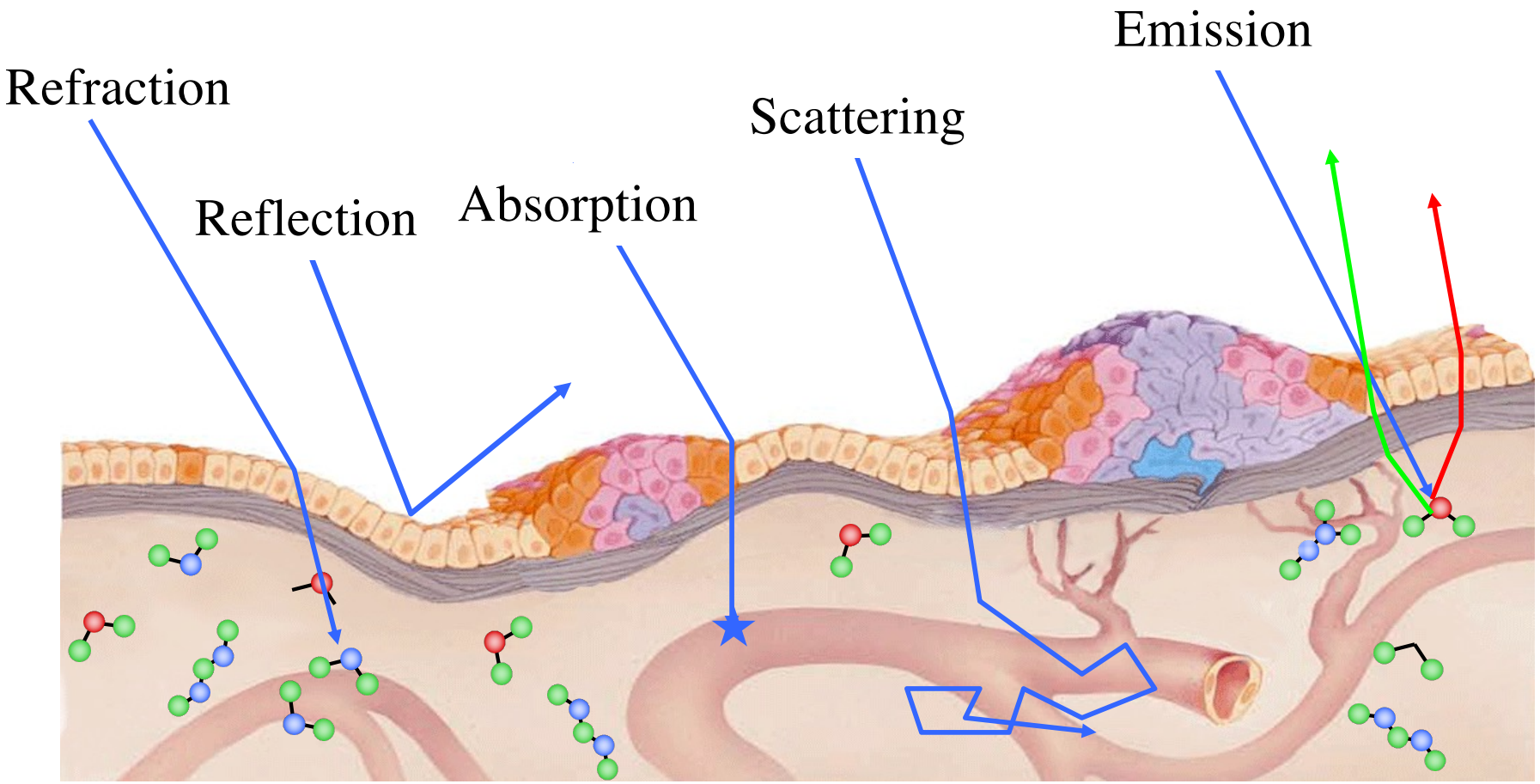
Assess all optical properties
non-invasively in **living tissue (in vivo)**

Difficulties

- Tissue is a complicated heterogeneous system
- Light penetration is limited (typ. several mm)

Light and Tissue

Types of interactions



Light and Tissue

Types of Interactions

- Reflection (Fresnel's law) $R = 1 - T = \frac{(n_1 - n_2)^2}{(n_1 + n_2)^2}$
- Refraction (Snell's law) $n_2 \sin(\Theta_2) = n_1 \sin(\Theta_1)$
- Scattering, Diffraction
- Absorption $I = I_0 e^{-\alpha(\lambda)z}$
  => Variation in Transmission (Beer's law)
- Phase shifts
- Emission

Light and Tissue

Optical properties and parameters

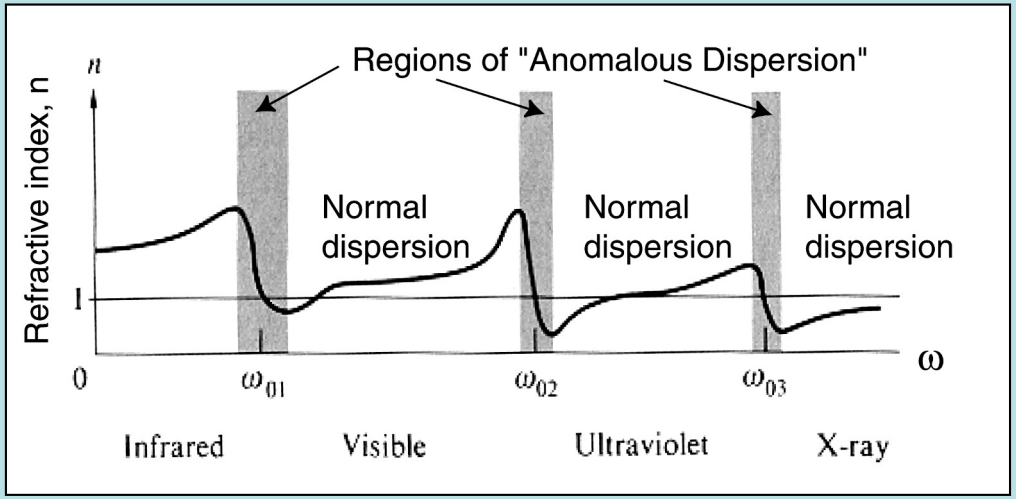
Interaction	Parameter		Unit	
Refraction	Refractive index*	n	[-]	ratio of the light velocity in a vacuum to its velocity in the tissue
Absorption	Absorption coefficient*	μ_a	[mm^{-1}]	Inverse of the mean free path before photon absorption
Scattering	Scattering coefficient*	μ_s	[mm^{-1}]	Inverse of the mean free path between photon scattering
Anisotropy	Anisotropy factor*	g	[-]	describes the distribution of scattering
Reduced scattering		$\mu_s' = \mu_s(1-g)$		
Effective attenuation		$\mu_{eff} = (3\,\mu_a(\mu_a + \mu_s'))^{1/2}$ inverse if the penetration depth if $\mu_a << \mu_s'$		

*fundamental microscopic parameters

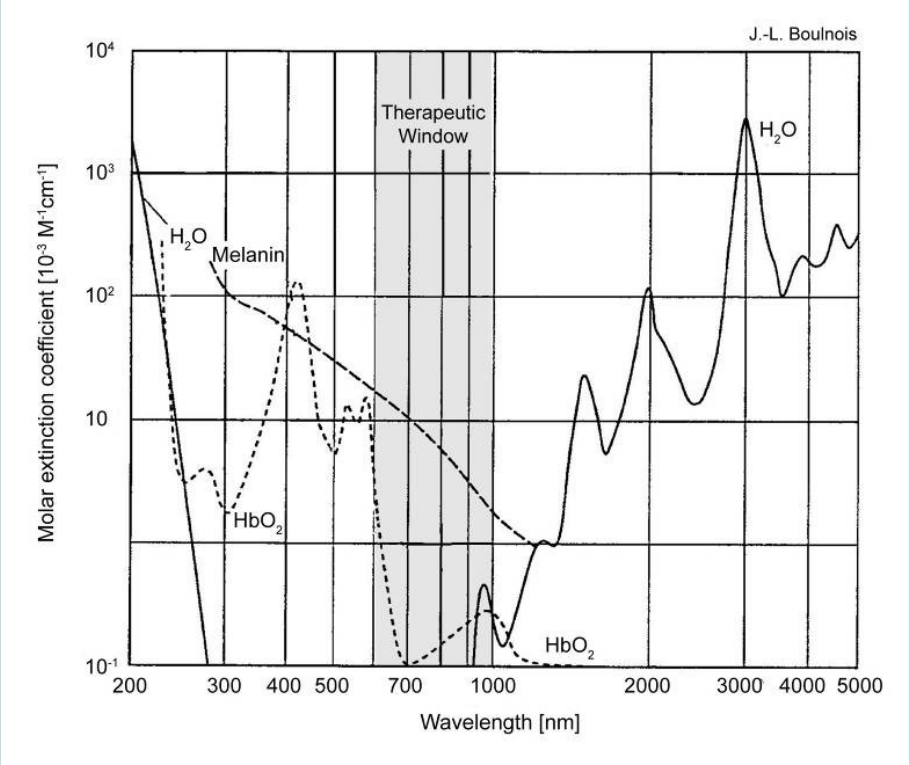
Light and Tissue
Optical properties and parameters

All optical parameters of biological tissue depend on the wavelength of the light

Refractive index

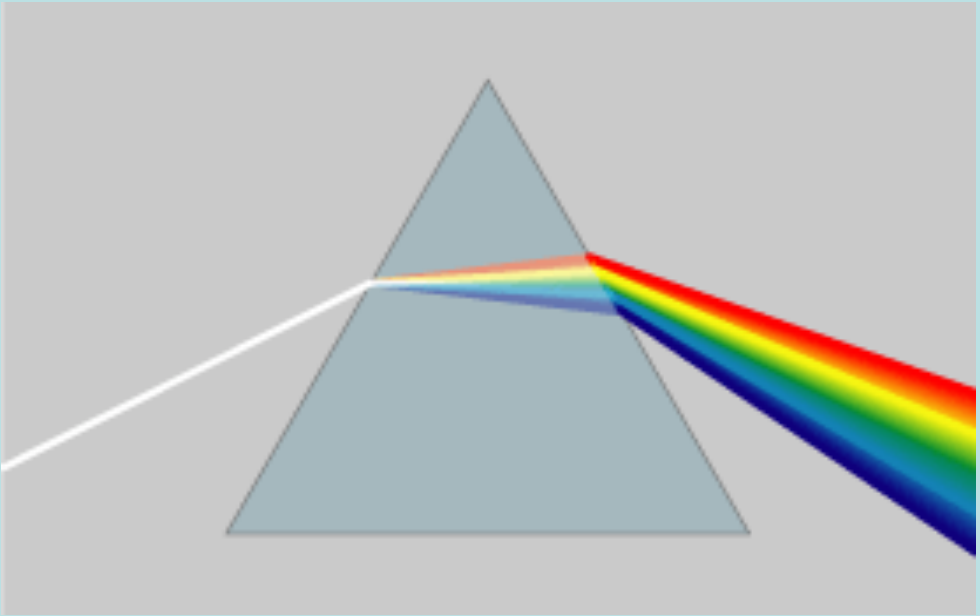


Absorption



Dispersion

= the wavelength dependence of the refractive index



Best known example:
Diffraction of visible light by a prism

$$n_2 \sin(\Theta_2) = n_1 \sin(\Theta_1)$$

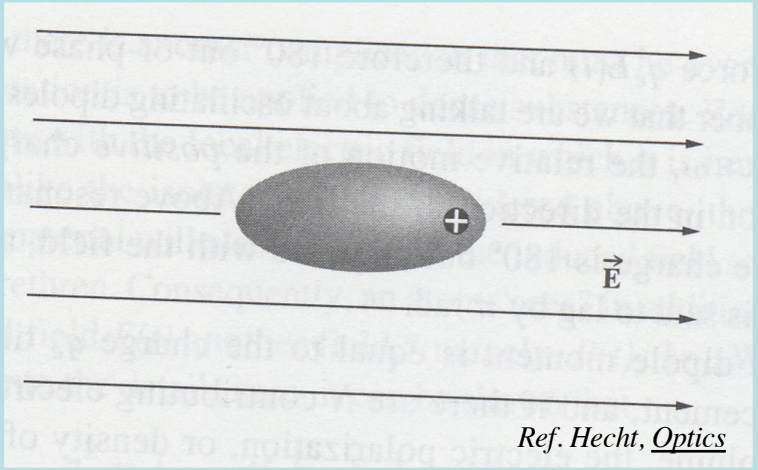
What is the origin of the wavelength/frequency dependence of n ?

Microscopic Origin of Dispersion

Distortion of an Electron Cloud in response to an applied **E**-field

- the internal charge distribution is modified

➡ generation of electric dipole moments



Ref. Hecht, Optics

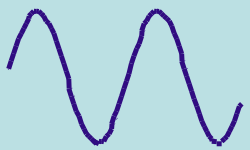
Incident harmonic EM wave:

- time-varying forces and torques act upon internal charge distribution

➡ generation of oscillating dipoles

Microscopic Origin of Dispersion

When excited by the light wave, the electron will oscillate at the driving frequency, and emit light at that frequency.



Incident light

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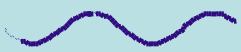

Emitted light

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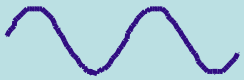


Transmitted light

The crucial issue is the relative phase of the incident light and this reemitted light …

Microscopic Origin of Dispersion

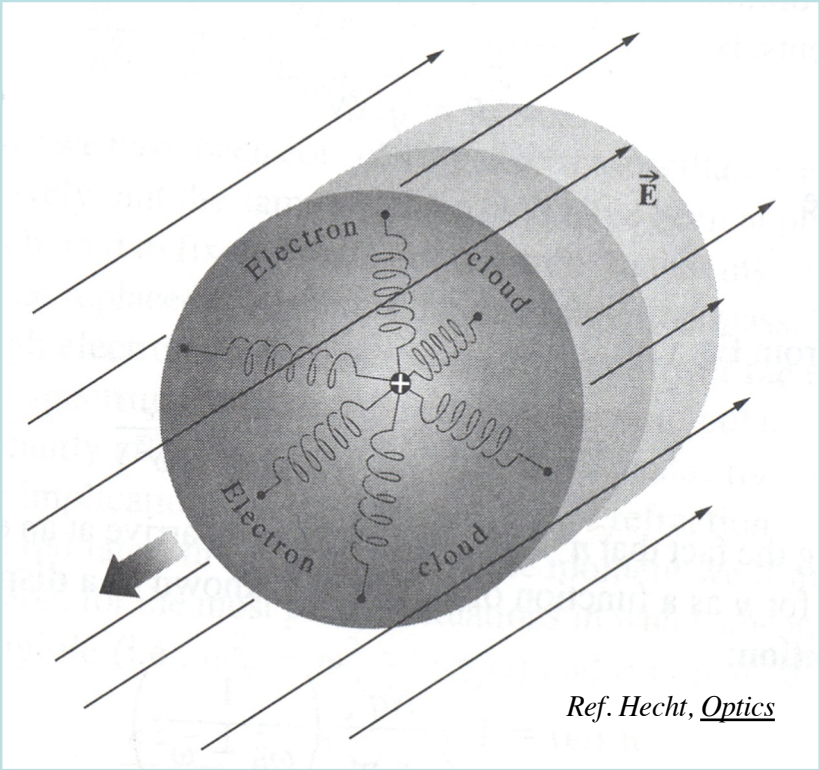
Consider the electron to be elastically bound to the nucleus, like a harmonic oscillator with a natural or resonant frequency ω_o.

$\vec{E}$ ⟵ ⟶ $\vec{F}$

driving force restoring force

- for short replacements x, F should be linear: $F = -k\,x$

- resonant frequency $\omega_0 = \sqrt{\frac{k}{m_e}}$



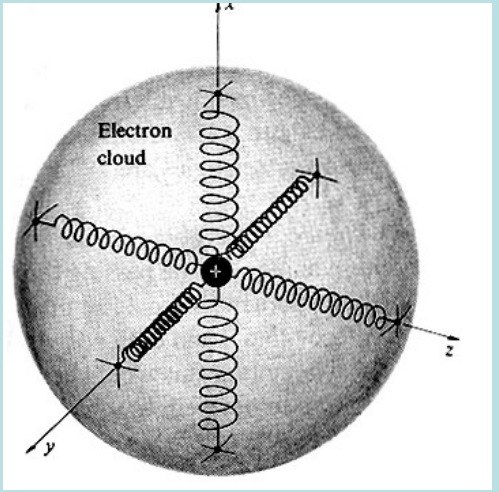
Ref. Hecht, Optics

Important Note

For simplicity here, we are

- assuming that the electron cloud is elastically bound to the nucleus **isotropically**,

  *but in complex materials, the molecular "spring constant" can be different for different orientations (polarizations) of the **E**-field. This will lead to different indices of refraction for different **E**-field polarizations … "**birefringence**."*



Ref. Hecht, Optics

- only considering the **harmonic** response of the electron cloud to the driving force of the incident **E**-field.

  *Assuming such a linear response is correct, if the displacements induced are small. Large **E**-fields (present in high intensity laser pulses) can induce large displacements, and non-linear restoring forces. In this case, there are higher order, anharmonic terms to consider. This is the domain of **Nonlinear Optics**.*

Microscopic Origin of Dispersion

Consider an electron on a spring with position $x_e(t)$, and driven by a light wave, $E_0 \exp(-i\omega t)$:

$$m_e \frac{d^2 x_e}{dt^2} + m_e \omega_0^2 x_e = e E_0 e^{-i\omega t}$$

m_e = electron mass
ω_0 = Resonant frequency
e = electron charge

mass • acceleration

restoring force

driving force

(see Hecht, Addison Wesley, 2000)

The solution is: $$x_e(t) = \left[\frac{(e/m_e)}{\omega_o^2 - \omega^2}\right] E_o e^{-i\omega t}$$

The amplitude of oscillation is frequency dependent, with the maximum displacement occurring at the resonant frequency, ω_o.

Microscopic Origin of Dispersion

A more realistic model is a damped forced oscillator, a harmonic oscillator experiencing a sinusoidal force and viscous drag (friction).

Add a viscous drag term: γ

Term for dumping force linear proportional to velocity of mass

$$eE_0 e^{-i\omega t} = m_e \frac{d^2 x_e}{dt^2} + m_e \omega^2 x_e + m_e \gamma \frac{dx_e}{dt}$$

The solution is now:

$$x_e(t) = \left[\frac{(e/m_e)}{\omega_o^2 - \omega^2 - i\omega\gamma} \right] E(t)$$

Microscopic Origin of Dispersion

The solution $$x_e(t) = \left[\frac{(e/m_e)}{\omega_o^2 - \omega^2 - i\omega\gamma}\right] E(t)$$

Can be rewritten as: $$x_e(t) = x_o e^{-i(\omega t - \alpha)}$$

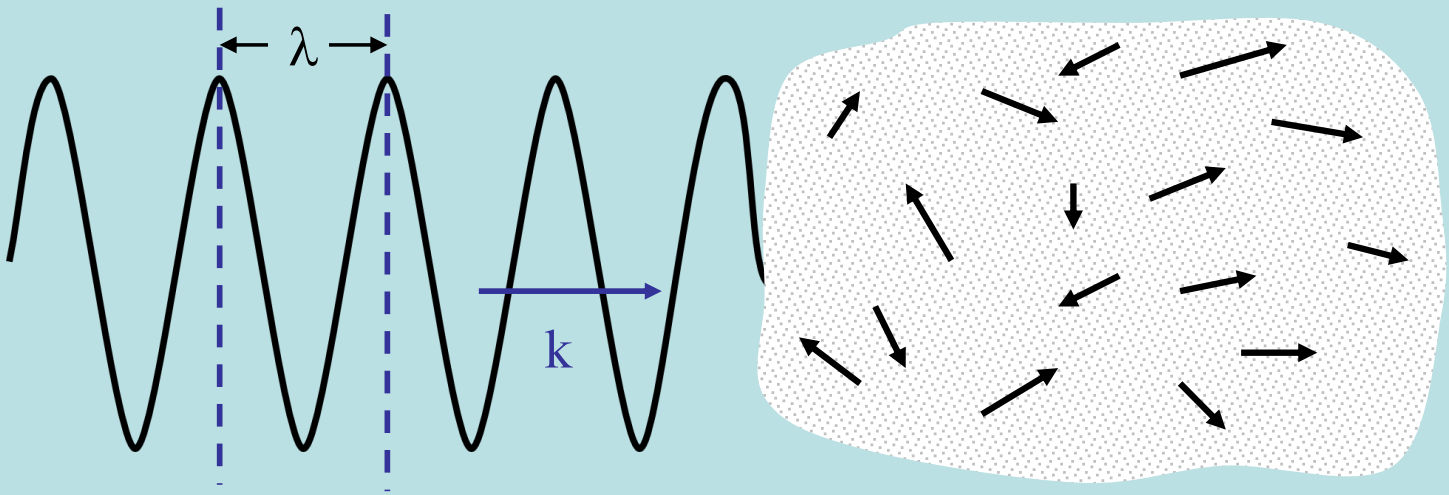
(see Hecht, Addison Wesley, 2000)

Where $$x_O = \frac{eE_O}{m_e} \frac{1}{\sqrt{(\omega_O^2 - \omega^2)^2 + \gamma^2\omega^2}}$$

and α is the phase lag $$\alpha = \arctan\left(\frac{\gamma\,\omega}{\omega_0^2 - \omega^2}\right)$$

The electron still oscillates at the driving frequency of the light wave, but with an *amplitude and a phase* that depend on the relative frequencies.

The incident light wave will induce a polarization in the medium



Polarization: $P(t) = N\, e\, x_e(t)$

where N is the number density of charges,
e is the charge per particle, and
$x_e(t)$ is the displacement of each charge vs. time.

It can be shown that the Electric Field in the Medium is given by: (see Hecht, Addison Wesley, 2000)

$$E(z,t) = E_0 \cdot e^{(-\alpha/2)z} \cdot e^{i(nkz-\omega t)}$$

Absorption causes attenuation of the field

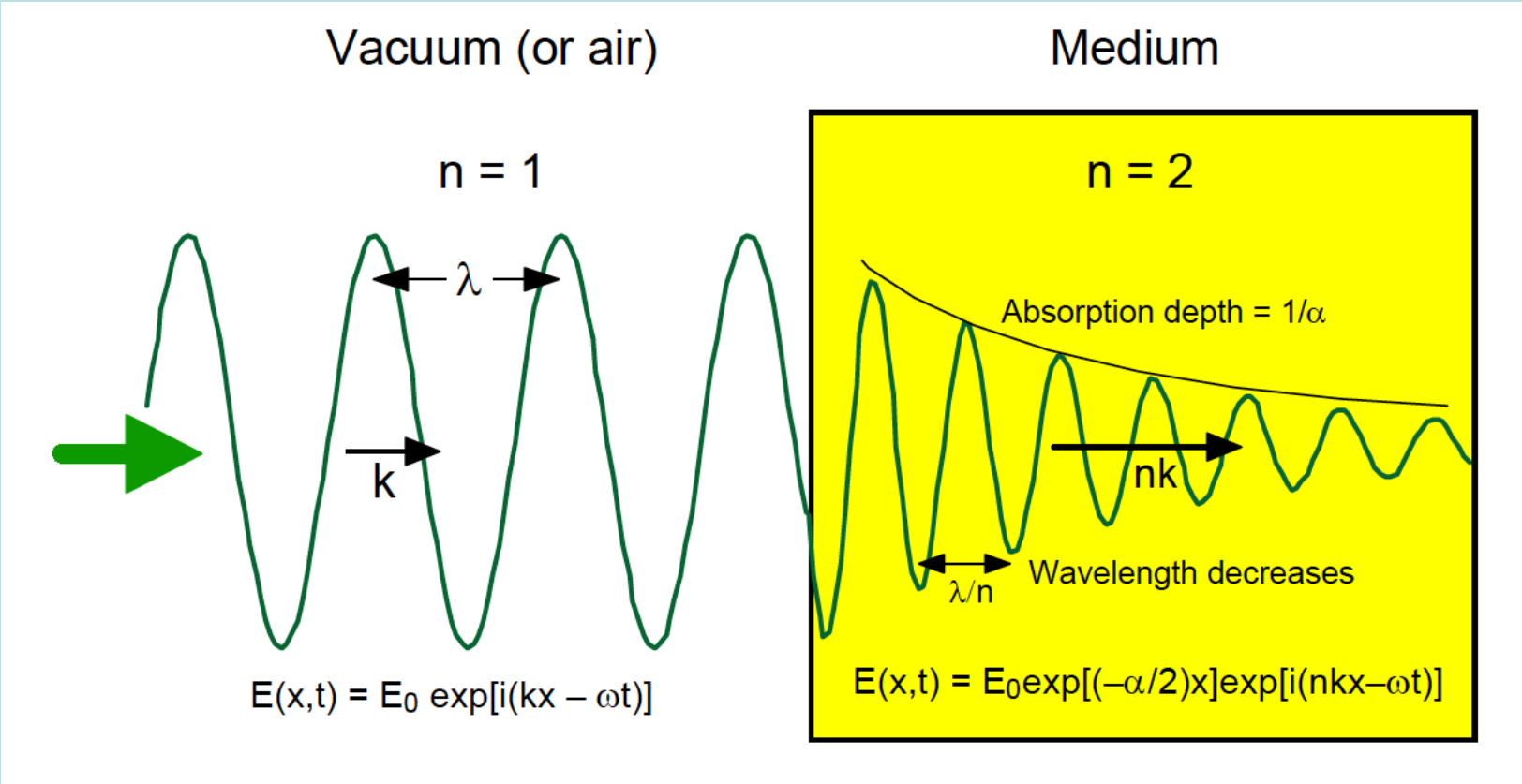
Refractive index changes the k-vector

$$\alpha = \frac{Ne^2}{4m_e\epsilon_o c_o} \frac{\gamma/2}{(\omega_o-\omega)^2+(\gamma/2)^2} \qquad n-1 = \frac{Ne^2}{4m_e\epsilon_o} \frac{\omega_o-\omega}{(\omega_o-\omega)^2+(\gamma/2)^2}$$

c_0 = speed of light in vacuum

... and undergo Absorption

The incident light wave will induce a polarization in the medium that will radiate and modify the propagating wave …



Typically, the speed of light, the wavelength, and the amplitude decrease

Electric Field in the Medium - Significance

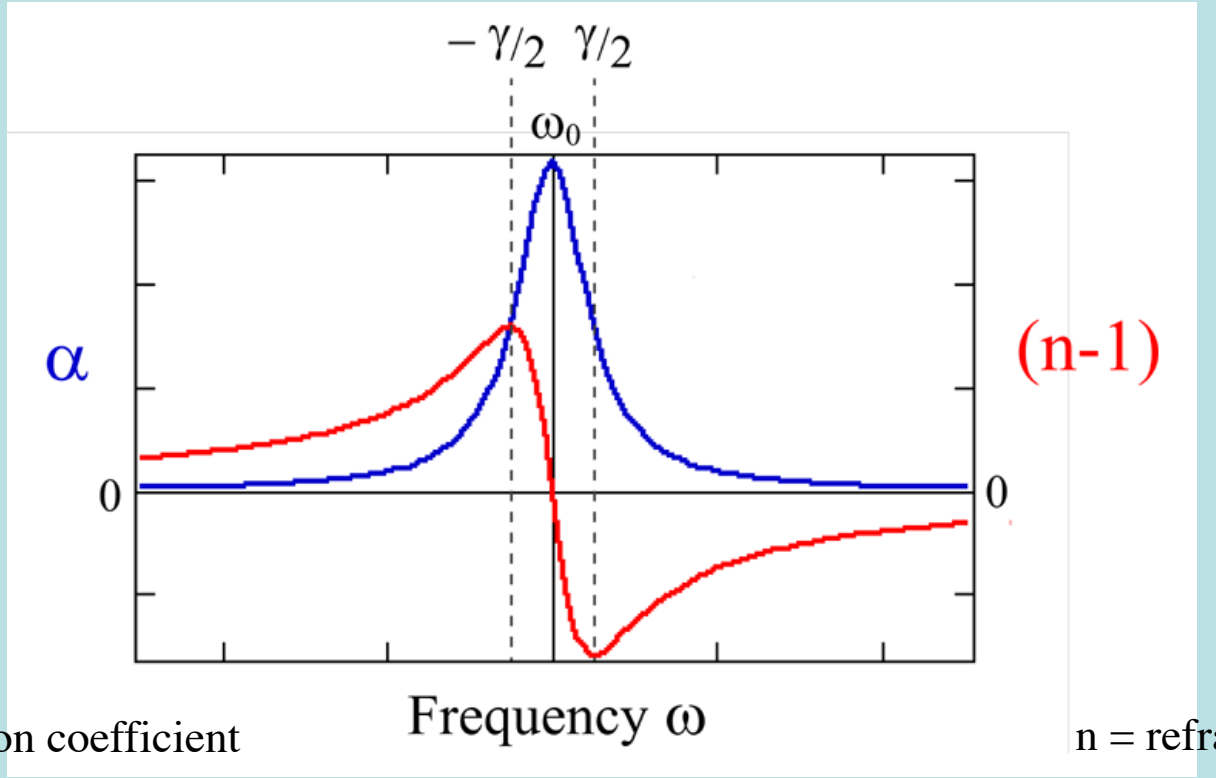
Electrical field $$E(z,t) = E_0(0) \cdot e^{(-\alpha/2)z} \cdot e^{i(n\,kz - \omega t)}$$

Refractive index $$n - 1 = \frac{Ne^2}{4m_e\varepsilon_0} \frac{\omega_0 - \omega}{(\omega_0 - \omega)^2 + (\gamma/2)^2}$$

Refractive index and wavelength/frequency correspondance:

$\omega_0 >> \omega$	➡ n hardly depends on λ	➡ Transparency/colorless
$\omega \rightarrow \omega_0$	➡ n increases with λ	➡ Normal Dispersion
$\omega = \omega_0$	➡ E is dominated by absorption + viscous drag	➡ Anomalous Dispersion

Refractive Index and Absorption Coefficient



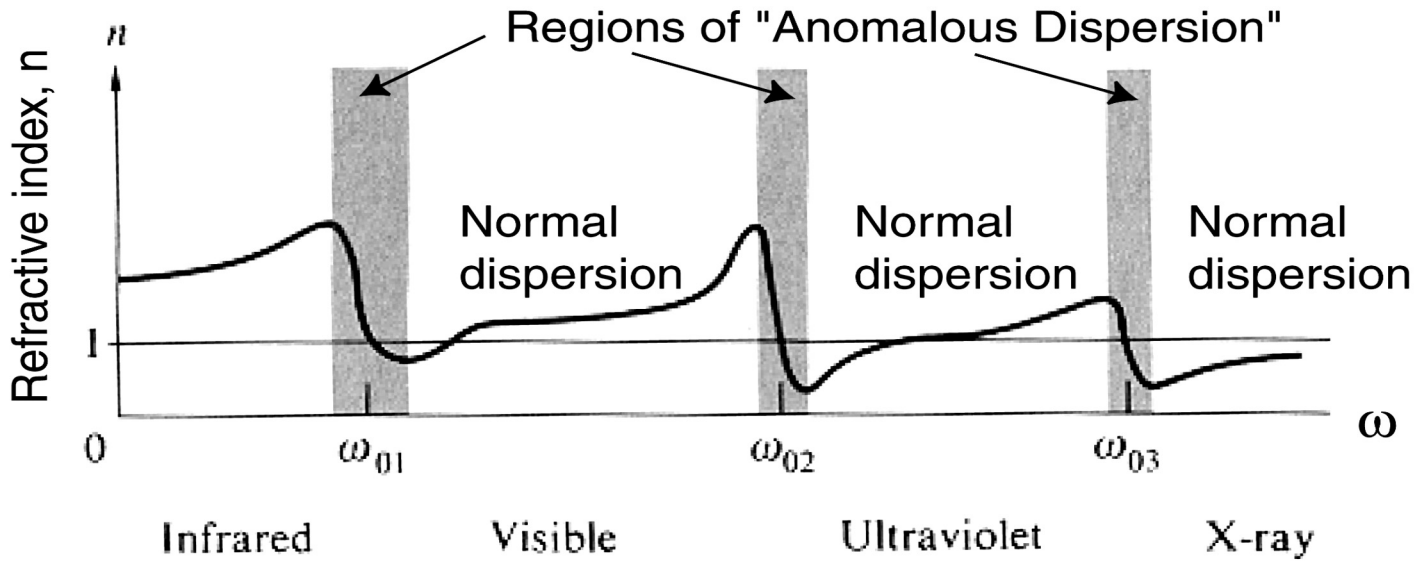
α = absorption coefficient

n = refractive index

Note:

- the region between (-γ/2) and (γ/2) corresponds to the region of anomalous dispersion

Normal and anomalous Dispersion

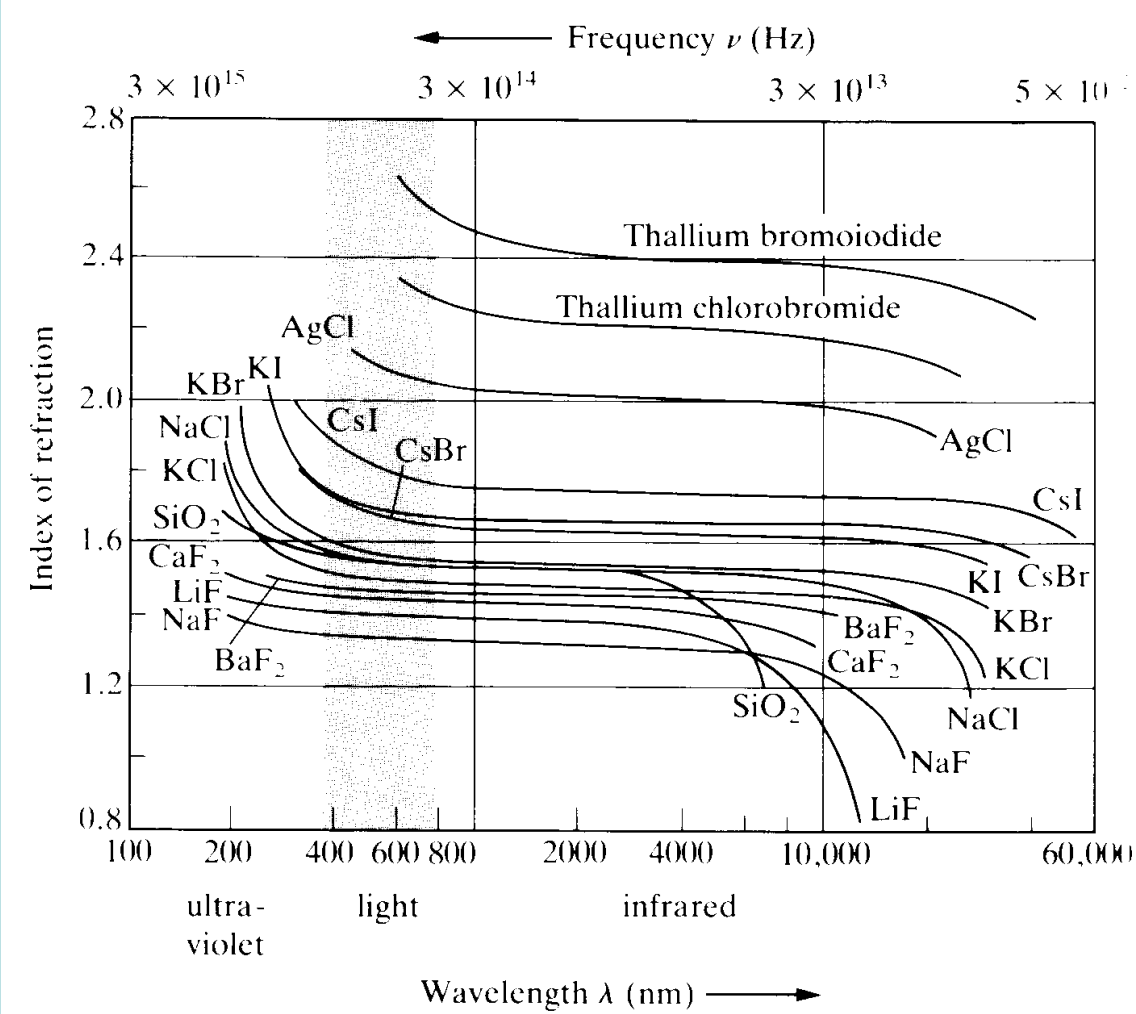


Ref. Hecht, Optics

IR: Vibrational and rotational resonances
Visible and UV : Electronic resonances
X-rays: "Inner-shell" electronic resonances

n increases with frequency, except in "anomalous dispersion" regions, where there is absorption.

Dispersion: Refractive Index vs. Wavelength



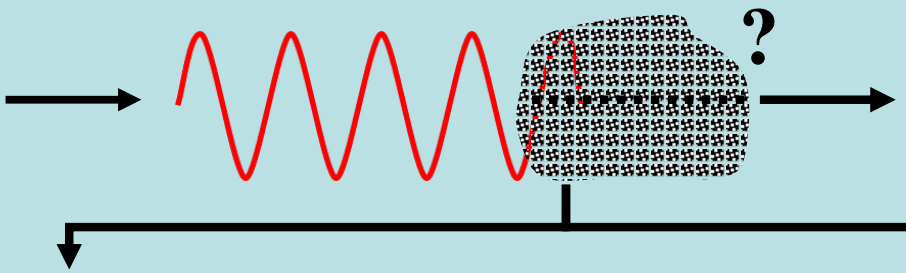
Index of refraction versus wavelength and frequency for several important optical crystals.

(Adapted from data published by The Harshaw Chemical Co.)

Microscopic Origin of Dispersion

A more quantum-based interpretation

Interaction of EM waves with atoms/molecules in a medium:



$E_{ph} < E_{electr(n, n+m)}$

Distorsion and Oscillation of electron cloud

Oscillating Dipole emitting light with same λ than incoming wave

$E_{ph} = E_{electr(n, n+m)}$

Absorption of photon

Photon energy transfered to atom

E_{ph}= Energy of incoming photon; $E_{electr(n,n+m)}$= Energydifference between two electronic states of the atom

Refractive Index values for Tissues (at 550 nm)

• Water	1.33
• Extracellular fluids, cytoplasm	1.35 – 1.38
• Whole tissues (brain, lung, aorta, …)	1.36 - 1.40
• Fatty tissues	1.45
• Tooth enamel	1.62
• Melanin	1.7

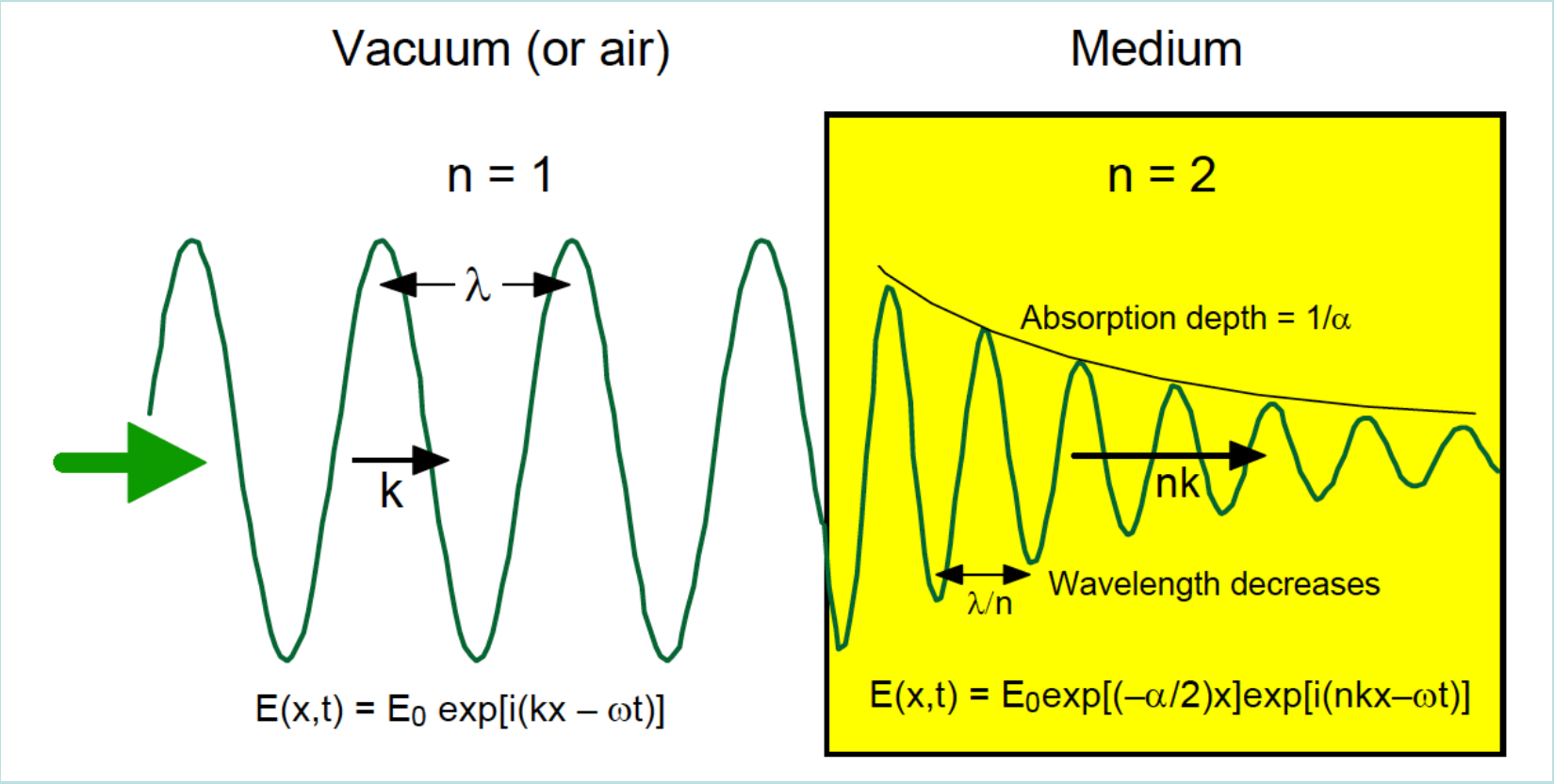
Refractive Index Values of Cell Components (at 550 nm)

Cell Component	Index
cytoplasm	1.35
cytoplasm	1.358-1.374
cortical cytoplasm	1.353-1.368
lipid	1.48
melanin	1.7
protein	1.50
dried protein	1.58
cytoplasm, rat liver cells	1.38
mitochondria, rat liver cells	1.40
mitochondria, rat liver	1.42
cytoplasm, hamster ovary cells	1.37

Ref: www.nmr.mgh.harvard.edu

Absorption Coefficient and the Irradiance

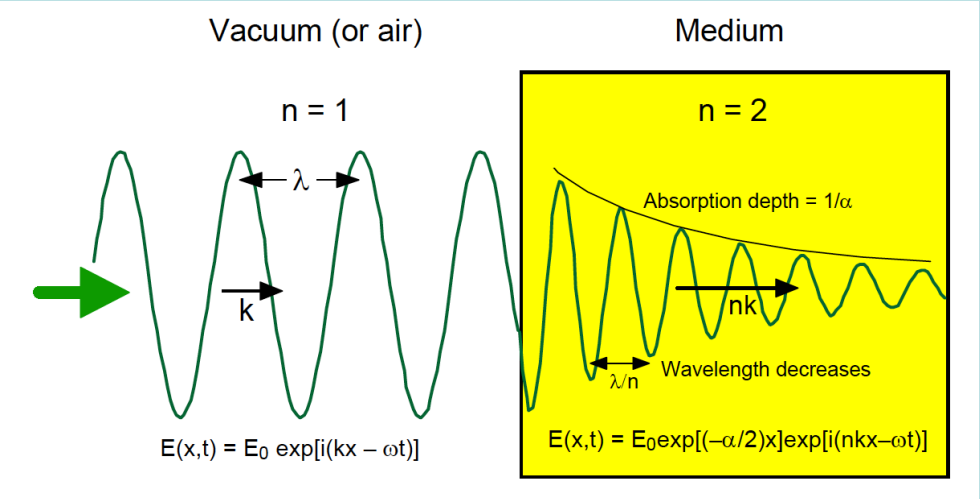
(Beer-Lambert Law)



The Irradiance $I(x) = |E(x,t)|^2 = I(0)\exp(-\alpha x)$

Absorption Coefficient and the Irradiance

(Beer-Lambert Law)



If you can measure …

- the incident irradiance *I(0)*

and

- the irradiance *I(x)* transmitted through a sample of known thickness x

then you can determine the absorption coefficient α of the sample

The Irradiance $I(z) = I_0 \exp(-\alpha x)$

ABSORPTION in TISSUE

Absorption

= Extraction of energy from light by matter

Diagnostic applications

Transitions between two energy levels of a molecule that are well defined at specific wavelengths could serve as spectral fingerprint of the molecule

- Various types of Chromophores (light absorbers) in Tissue
- Tumor detection & other physiological assessments (e.g. pulse-oxymetry)

Therapeutic applications

Absorption of energy is the primary mechanism that allows light form a source (laser) to produce physical effects on tissue for treatment purpose

- Lasik Eye Surgery (**L**aser **A**ssisted in **si**tu **K**eratomileusis)
- Tatoo Removal
- PDT
- vascular applications of laser

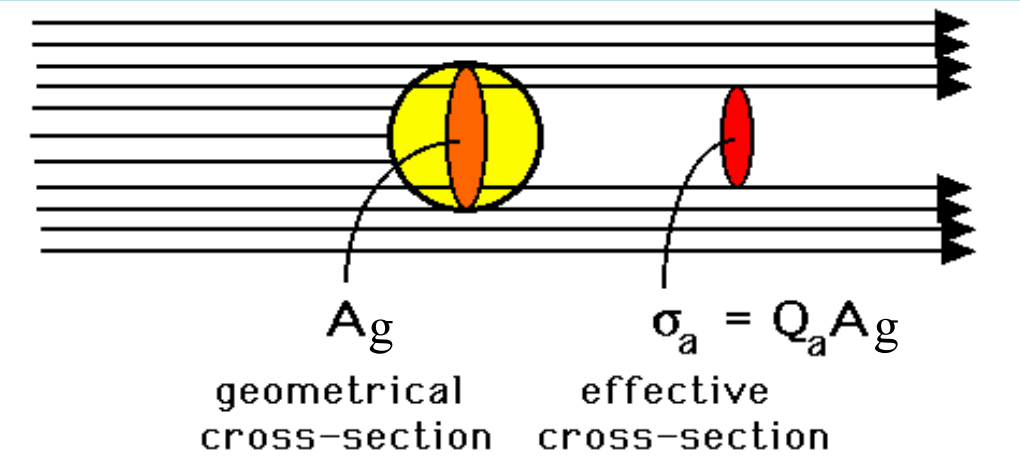
Metrics for Absorption

Absorption Cross-section σ_a [m^2]

- Consider a chromophore idealized as a sphere with a particular geometrical size.
- Consider that this sphere blocks incident light and casts a shadow, which constitutes absorption.
- The size of absorption shadow = absorption cross-section

$$\sigma_a = Q_a \cdot A_g$$

Q_a: absorption efficiency
A_g: geometrical cross-section

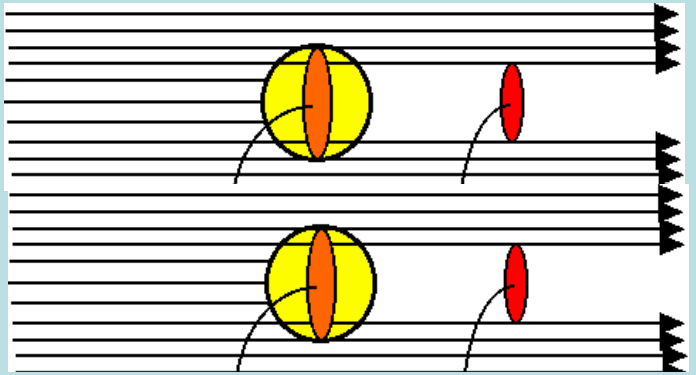


Metrics for Absorption

Absorption coefficient μ_a [cm^{-1}]

- describes a medium containing many absorbing particles and is defined as:

$$\mu_a = N_a \cdot \sigma_a$$



σ_a: the effective cross-section [m^2]

N_a: absorbers per cm^3 [m^{-3}] (= a volume density)

Assumptions:

- Cross section is independent of relative orientation of the impinging light and absorber
- uniform distribution of identical absorbing particles

➡ μ_a: Absorption cross-sectional area per unit volume of medium

Metrics for Absorption

Absorption mean free path l_a [m]

- Represents the average distance a photon travels before being absorbed

$$l_a = \frac{1}{\mu_a}$$

Absorption Fundamentals

Transmission coefficient T [--]

Absorbance A [--]

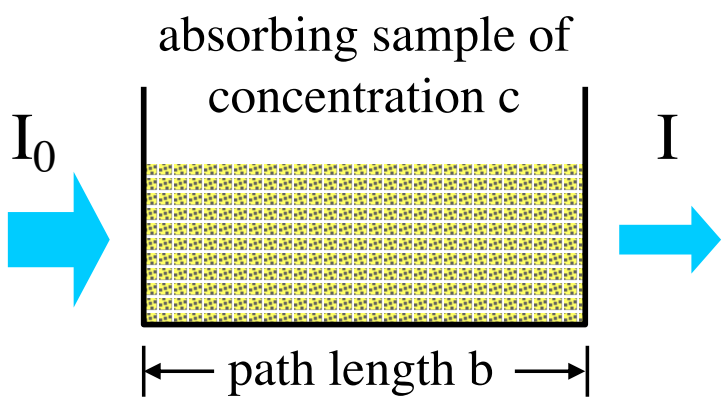
Transmission coefficient T

$$T = \frac{I}{I_o}$$

Absorbance A

(attenuation or optical density)

$$A = -\log(T) = \log\left(\frac{I_o}{I}\right)$$



How does μ_a relates with the absorbance and transmission ?

Connection between T/A and μ_a

An absorbing medium is characterized by the absorption coefficient μ_a, the transmission coefficient T, and the absorbance A.

Are they related?

→ Lambert – Beer (-Bouguer) Law:

describes the effect of either thickness or concentration of the sample on absorption

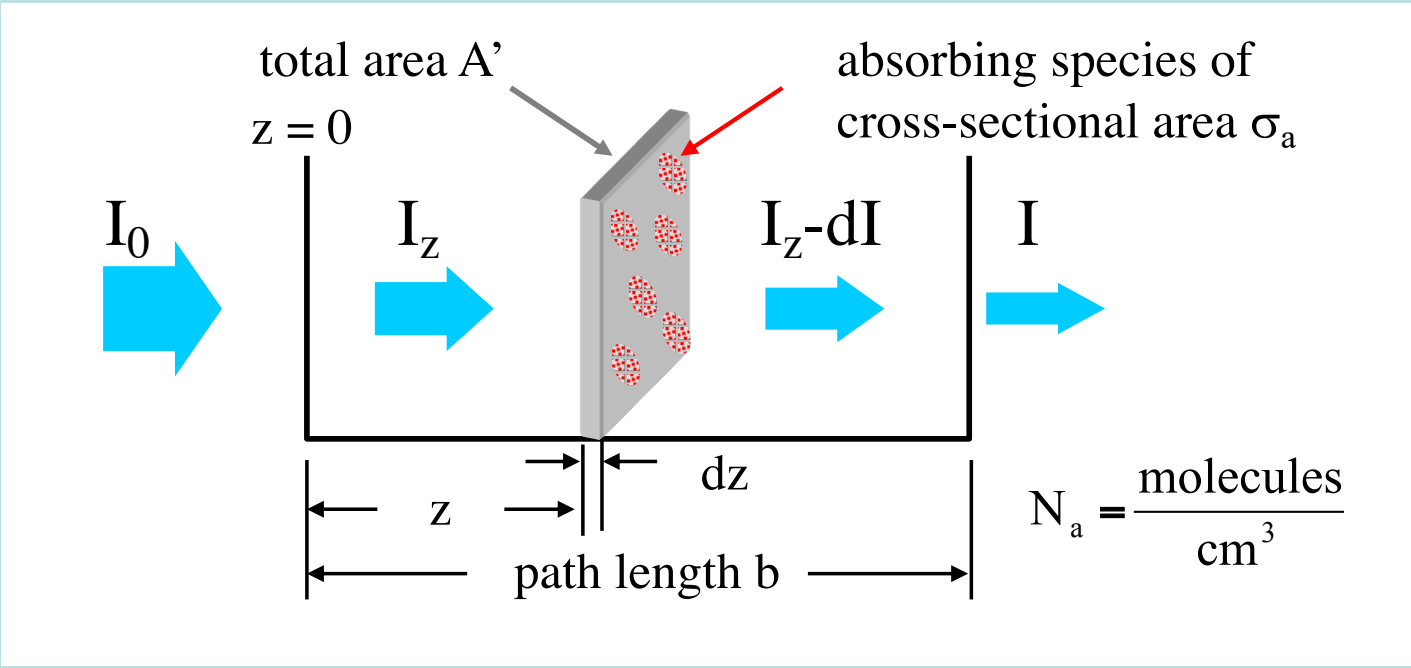
$$I = I_o e^{-\mu_a \cdot b}$$

Pierre Bouguer (1698–1758)
Johann Heinrich Lambert (1728–1777)

$$A = \varepsilon\, c\, b$$

August Beer (1825–1863)

Lambert-Beer Law



σ_a= absorption cross-sectional area [cm^2]

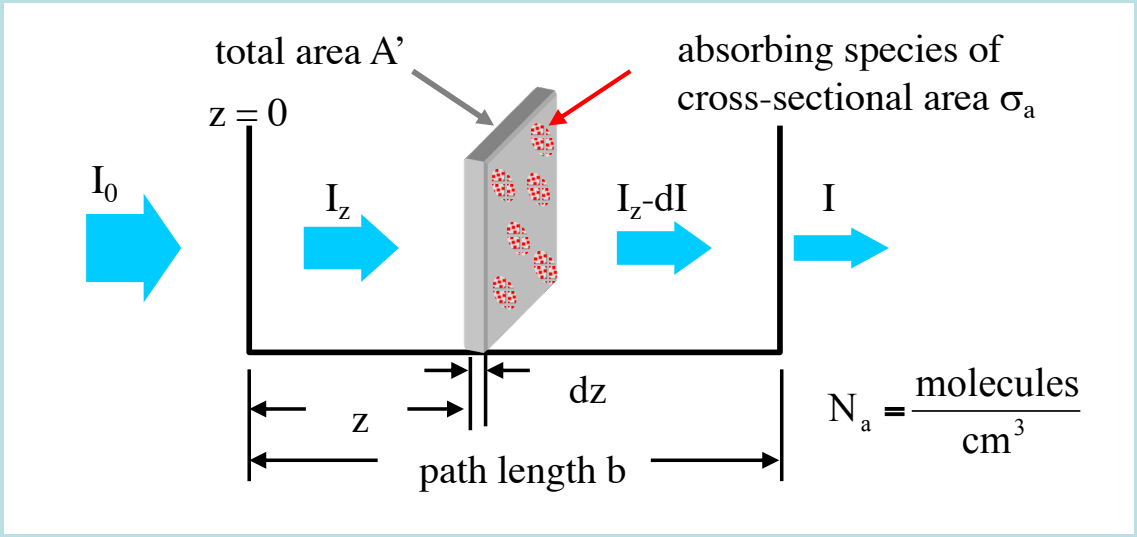
I_o = Intensity entering the sample at z = 0 [W/cm^2]

I = Intensity of light leaving the sample

I_z = Intensity entering the infinitesimal slab at z

dI = Intensity absorbed in the slab

Lambert-Beer Law



Total opaque area on the slab due to absorbers

$$\sigma_a \cdot N_a \cdot A' \cdot dz$$

Number of absorbers in the slab volume

Loss of intensity

$$dI = -\sigma_a \cdot N_a \cdot I(z) \cdot dz \quad \Rightarrow \quad \int_{I_o}^{I} \frac{dI}{I(z)} = -\int_0^b \sigma_a \cdot N_a \cdot dz$$

Fraction of photons absorbed

$$-\ln\left(\frac{I}{I_o}\right) = \sigma_a \cdot N_a \cdot b$$

Lambert-Beer Law

The concentration (molarity) is given by $c = N_a / N_A$ with N_A= Avogadro's constant

(1) $$c\left[\frac{mol}{cm^3}\right] = N_a\left[\frac{molec}{cm^3}\right] \Big/ \left(6.023\cdot 10^{23}\,\frac{molec}{mol}\right) \Leftrightarrow \quad N_a = 6.023\cdot 10^{23}\cdot c \quad \left[cm^{-3}\right]$$

moreover

(2) $$\log(x) \overset{(*)}{=} \frac{\ln(x)}{\ln(10)} = \frac{1}{2.303}\cdot\ln(x)$$

(*) $\log_b(x) = \frac{\log_k(x)}{\log_k(b)}$

- $\ln(x) = \log_e(x)$
- $\log(x) = \log_{10}(x)$

inserting (1) and (2) in $$-\ln\left(\frac{I}{I_o}\right) = \sigma_a \cdot N_a \cdot b$$

$$-\log\left(\frac{I}{I_o}\right) = \frac{1}{2.303}\cdot\sigma_a\cdot N_a\cdot b = \frac{N_A\cdot\sigma_a}{2.303}\cdot c\cdot b = \varepsilon\cdot c\cdot b = A$$

ε = Molar Extinction Coefficient [$cm^2\,mol^{-1}$] (Measure of 'Absorbing Power' of species)

Lambert-Beer Law

$$(3)\quad -\log\left(\frac{I}{I_o}\right) = \varepsilon \cdot c \cdot b \quad \Leftrightarrow \quad \frac{I}{I_0} = 10^{-\varepsilon \cdot c \cdot b} = \boxed{10^{-A} = T}$$

Correlation Absorbance-Transmission

$$(4)\quad \ln\left(\frac{I}{I_o}\right) = \ln\left(10^{-\varepsilon \cdot c \cdot b}\right) = -2.303 \cdot \varepsilon \cdot c \cdot b = -\sigma_a \cdot N_a \cdot b = -\mu_a \cdot b$$

$$\boxed{\mu_a = 2.303\ \varepsilon\ c}$$

Correlation
Absorption coeff.- Extinction coeff.

- By measuring the Transmission or the Absorbance for a given c, we can obtain ε → usually ex vivo
- If ε is known, and if we can measure μ_a in vivo, we can quantify the concentration of the chromophores

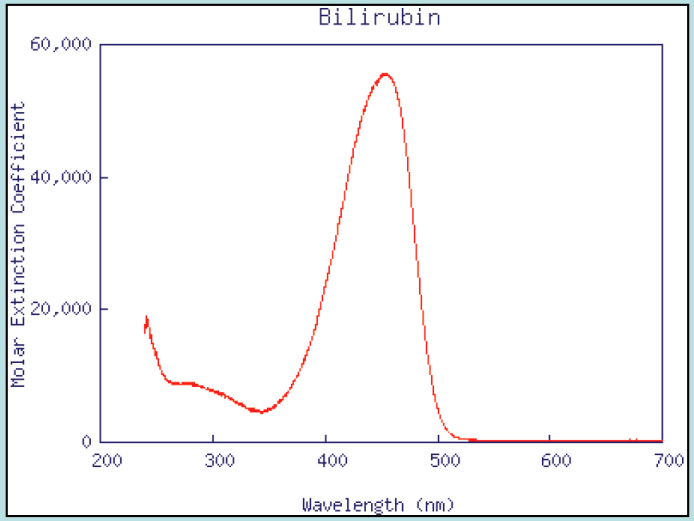
Limitation of Lambert-Beer's law

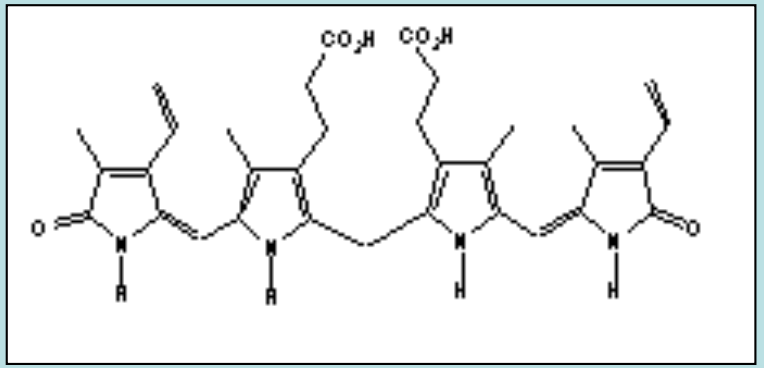
The Lambert-Beer's law is limited by:

- deviations in absorptivity at high concentrations (>0.01M)
- scattering of light due to particulates in the sample
- fluorescence or phosphorescence of the sample
- changes in refractive index at high analyte concentration
- shifts in chemical equilibria as a function of concentration
- non-monochromatic radiation, deviations can be minimized by using a relatively flat part of the absorption spectrum such as the maximum of an absorption band
- stray light

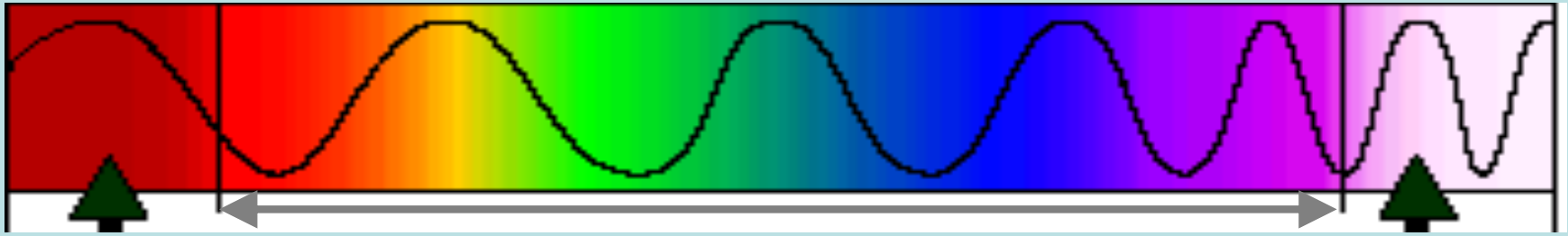
Bilirubin Example

- diameter is about 1 nm
- at 460 nm, ε=53846 [$cm^{-1}M^{-1}$]
- typical jaundiced neonates serum bilirubin concentration is 10 mg/dl
- Molar weight: 574.65 g/mole
- Concentration: 0.17 10^{-3} M
- What is μ_a ? (21 cm^{-1})
- Optical cross section: σ_a = ? (4.5x10^{-15} cm^2)
- Ratio optical versus geometrical cross section ? (0.6)





Absorbers in Tissue



NIR VISIBLE UV

NIR

- Hemoglobin
- Lipids
- Water

UV-VIS

- DNA
- Hemoglobin
- Lipids
- Structural protein*
- Electron carriers*
- Amino acids*

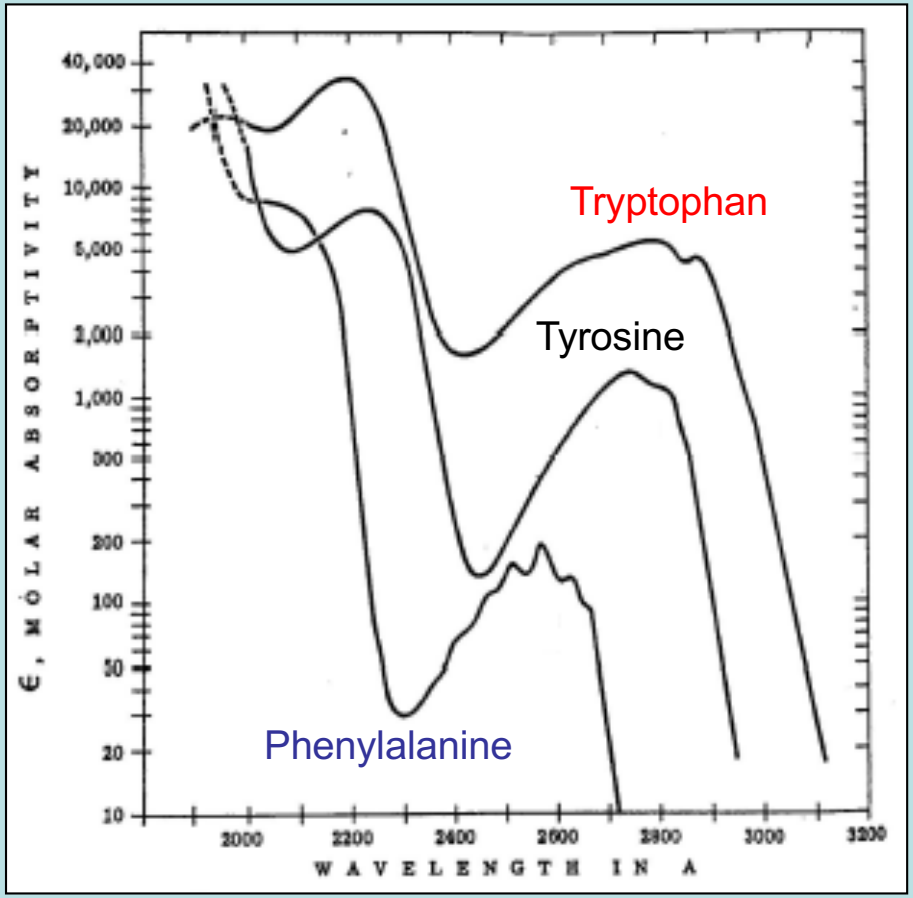
* Absorbers that fluoresce when excited in the UV-VIS

UV Absorption

Dominant absorbers in UV:
Protein, amino acid, fatty acid and DNA

- Protein is dominant 'non-water' constituent of all soft tissue (~ 30%)
- Absorption properties determined by peptide bonds and amino acid residues

Absorber	Absorption wavelength
Peptide	190 nm
Amino acids	210 nm – 220 nm 260 nm – 280 nm
DNA	≤ 320 nm



Large water absorption $\lambda < 180$ nm

Note!! The wavelength scale is in Å: 1Å= 0.1 nm

Molar extinction coefficients for Biologic Chromophores

Purine & Pyrimidine Bases and Derivatives

Molecule	λ [nm]	ε (x 10^{-3}) [cm^2 mol^{-1}]
Adenine (A)	260.5	13.4
Adenosine	259.5	14.9
Guanine (G)	275	8.1
Guanosine	276	9.0
Cytosine (C)	217	6.1
Thymine (T)	263.75	7.9

Molar extinction coefficients for Biologic Chromophores

Amino acids

Molecule	λ [nm]	ε (x 10^{-3}) [cm^2 mol^{-1}]
Tryptophan	280, 219	5.6, 47
Tyrosine	274, 222, 193	1.4, 8, 48
Phenylalanine	257, 206, 188	0.2, 9.3, 60
Histidine	211	5.9
Cystine	250	0.3

Molar extinction coefficients for Biologic Chromophores

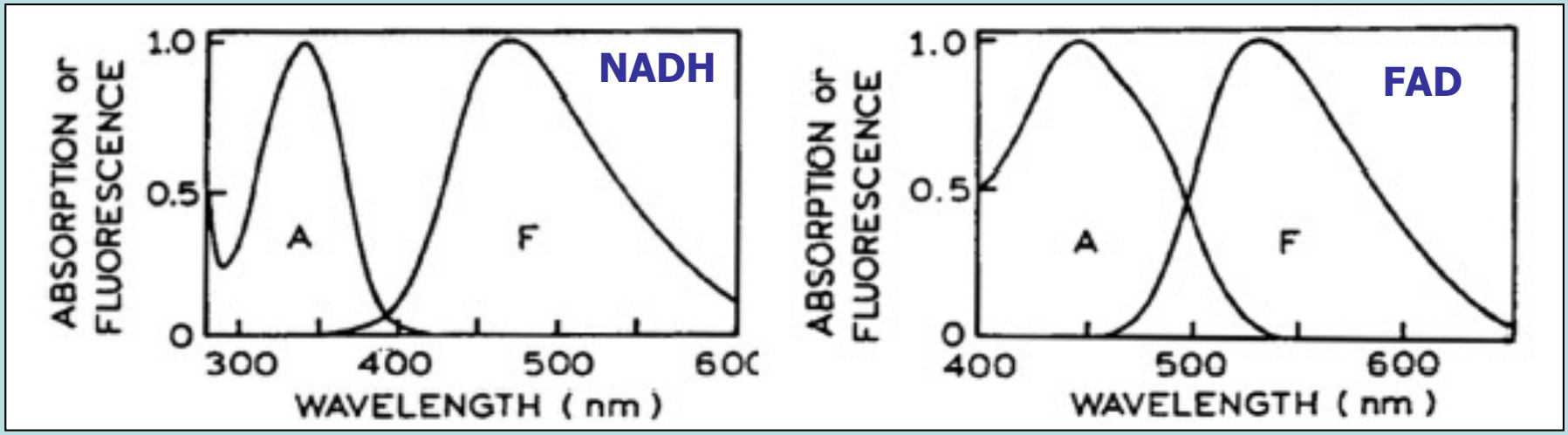
Respiratory Enzymes and nucleic acids

Molecule	λ [nm]	ε (x 10^{-3}) [cm^2 mol^{-1}]
NADH	340, 259	6.23, 14.4
NAD+	260	16.9
DNA, RNA	258, 260	6.6, 7.4

NADH: Nicotinamide- adenine dinucleotide

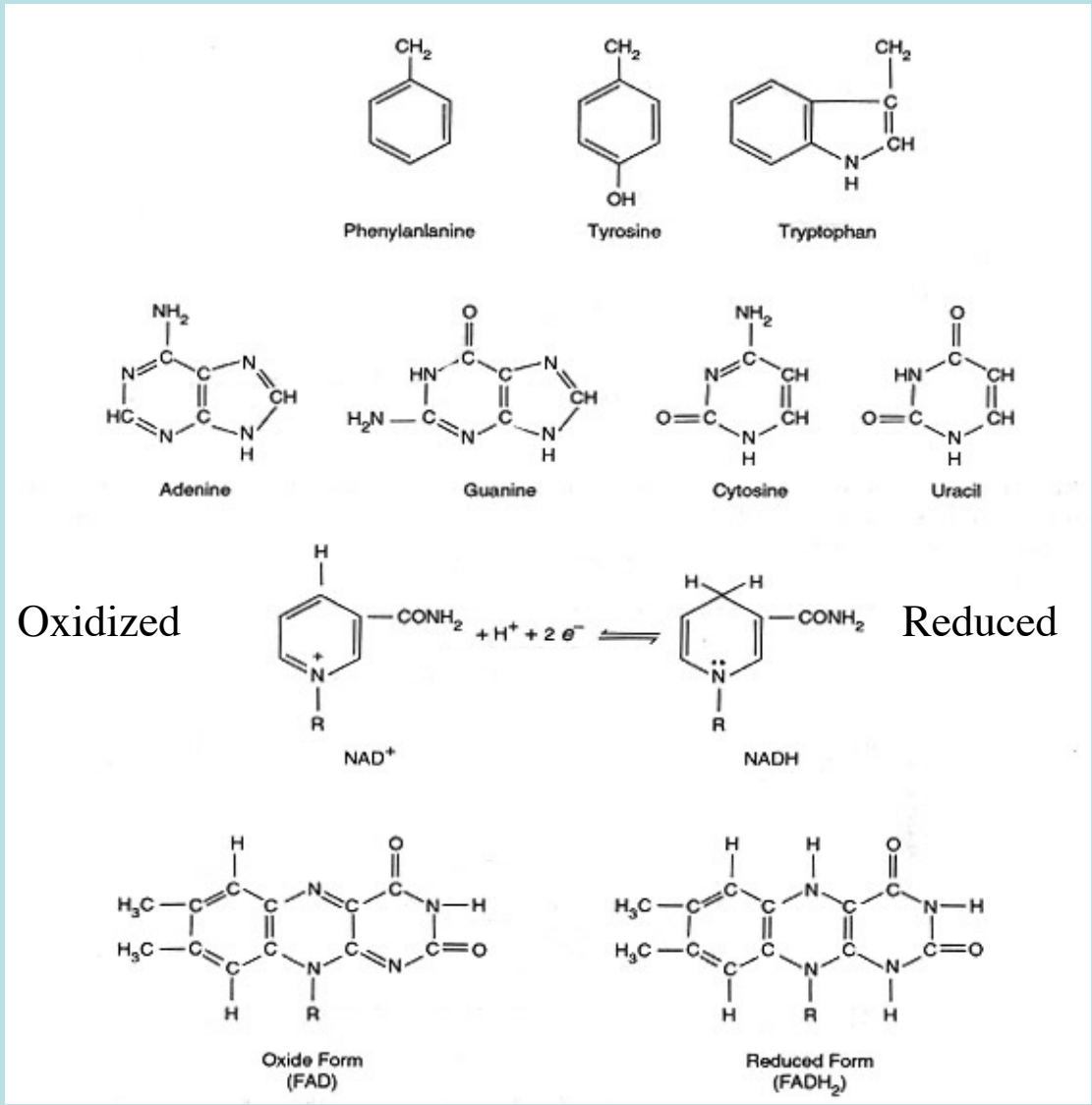
Respiratory Enzymes: NADH, FAD, Cytochrome a_3

- These enzymes play a key role in providing the proton-motive force necessary for oxidative phosphorylation
- If tissue is oxygen starved, [NADH] and [$FADH_2$] will be enhanced
- Reduced NADH concentration is indicative of high oxygen consumption and is characteristic of tumor tissue



- NADH (FAD) strongly fluoresce whereas NAD^+ ($FADH_2$) don't
- Cytochrome a_3 has a prominent absorption peak at λ = 840 nm

Biological Chromophores - Structures

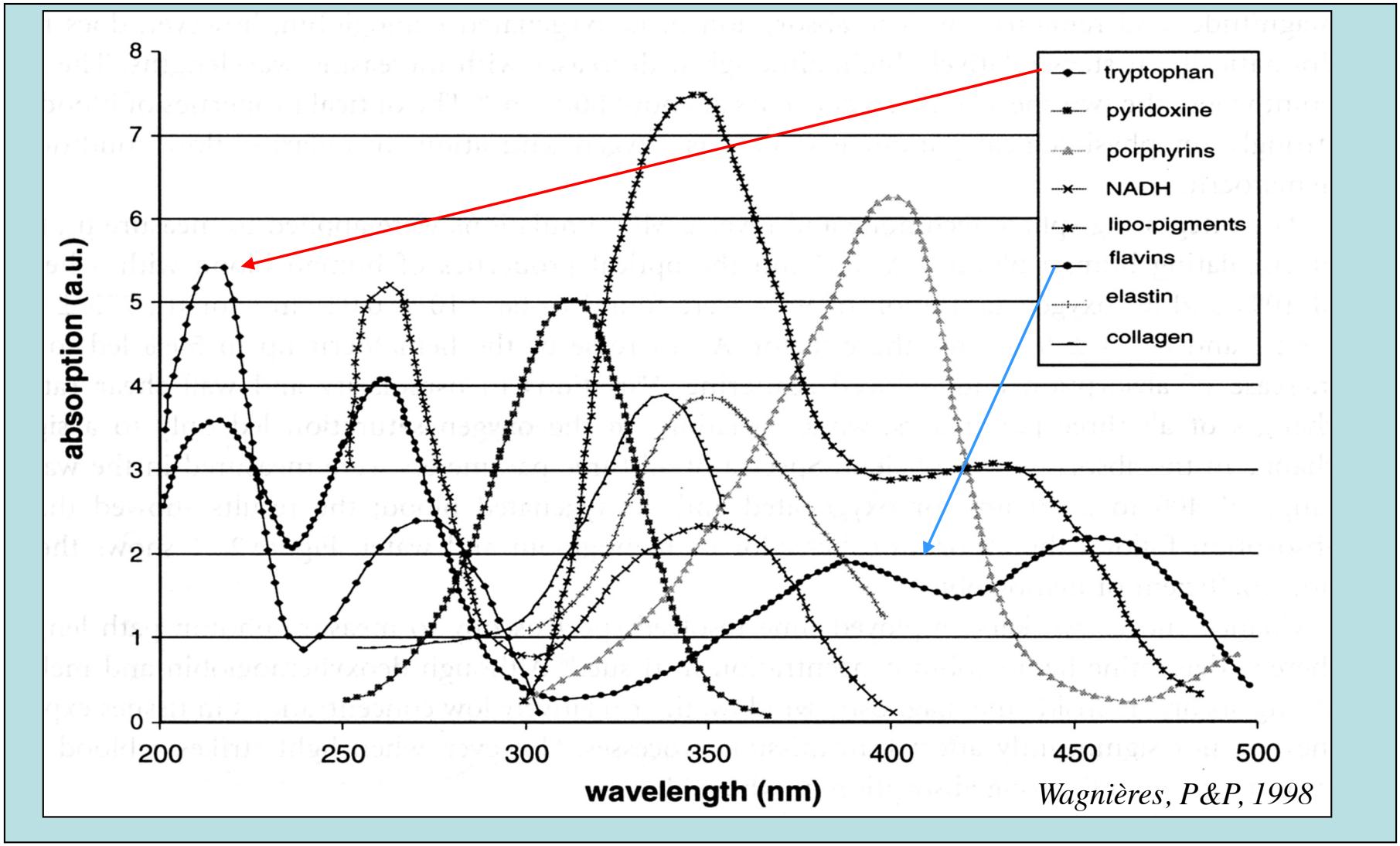


Oxidized

FAD: Flavin Adenine dinucleotide

Reduced

Absorption of various constituents in tissu

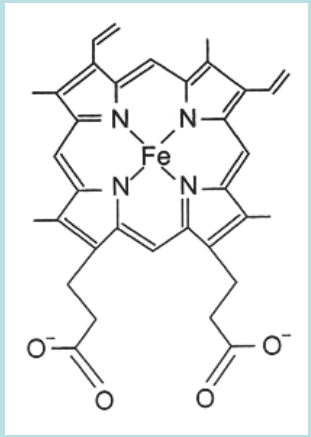


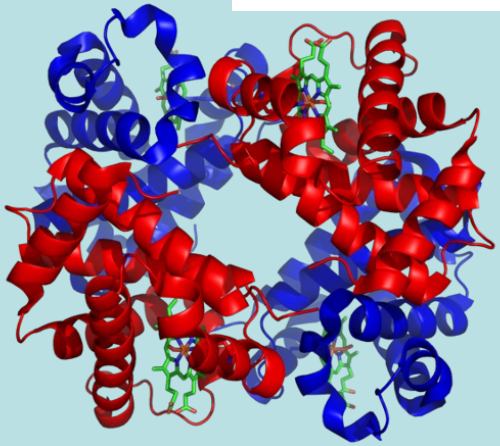
Visible and NIR Absorption

Main Absorbers at visible and NIR:

- Hemoglobin
- Lipid

Heme →





Hemoglobin

Hemoglobin

- Each hemoglobin has 4 heme (Fe^{2+}) sites to bind O_2
- Responsible for oxygen transport (HbO_2 and Hb)
- Oxygen saturation is an indicator of oxygen delivery and utilization as well as metabolic activity

Hemoglobin

Absorption peaks for

- HbO_2:

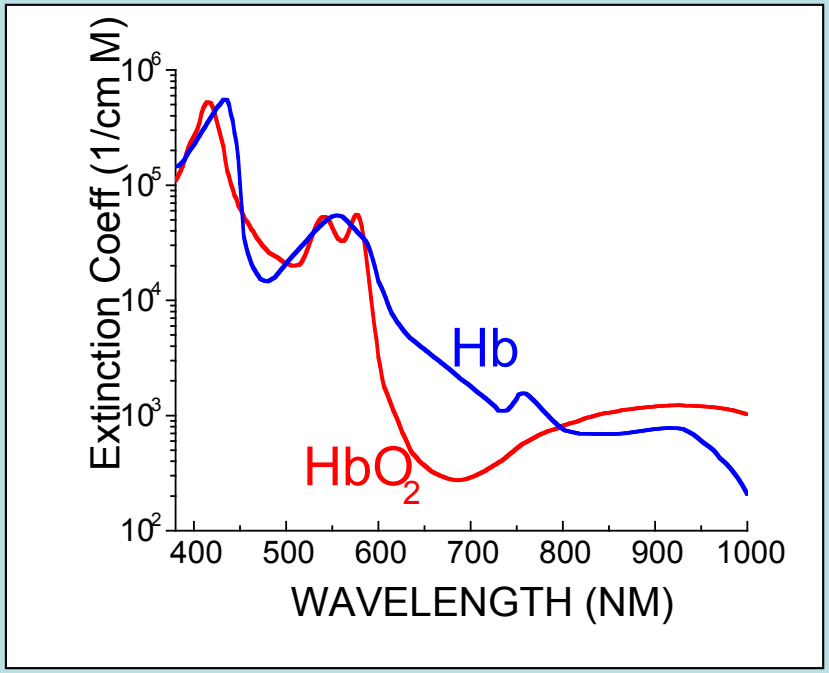
  418, 542, 577, and 925 nm

- Hb:

  430, 550, 758, 910 nm

Isobestic points*

547, 569, 586, and 798 nm



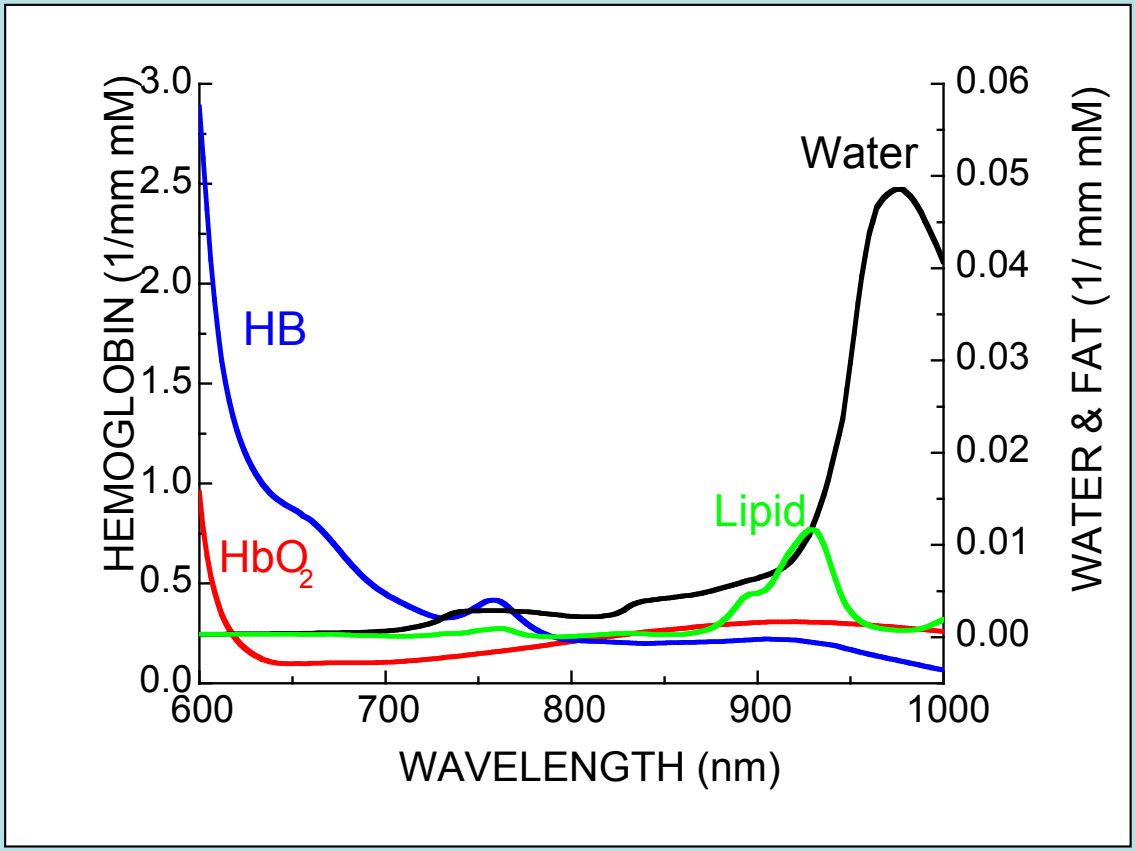
*(*) An **isosbestic point** is a specific wavelength at which two (or more) chemical species have the same extinction coefficients.*

Lipid (Fat)

- Important energy storage in the body

Tissue optics:

- Site-specific measurements of body composition
- Monitoring of physiological changes in female breast tissue

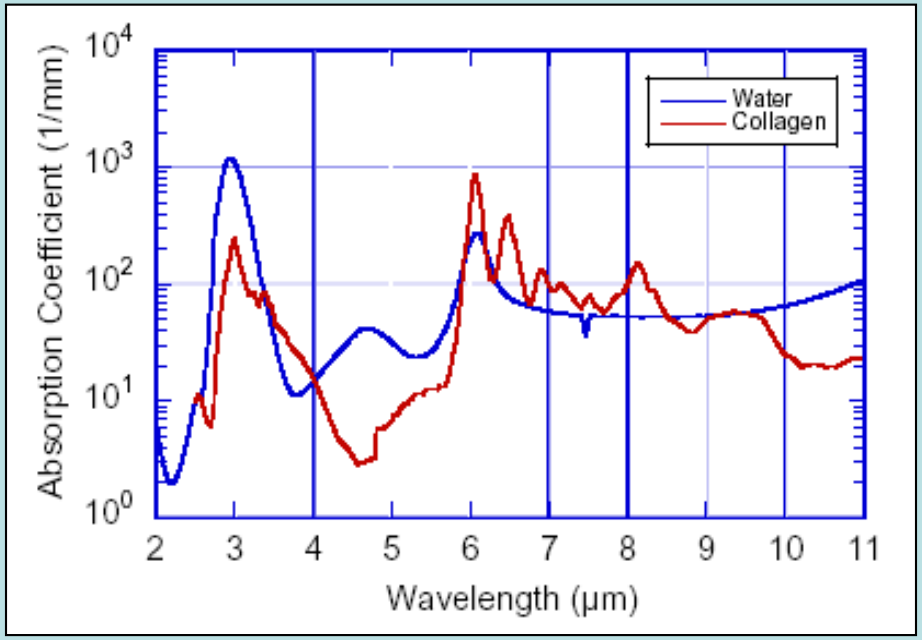


Lipids absorption probably due to C-H vibration mode overtones

Infrared Absorption

Many proteins have IR absorption peaks at 6.1, 6.45, and 8.3 μm due to amide excitation

- Penetration depth ≤ 10 μm in $\lambda = 6 - 7$ μm region



Water absorption peak at 0.96, 1.44, 1.95, 2.94 and 6.1 μm

Penetration depth

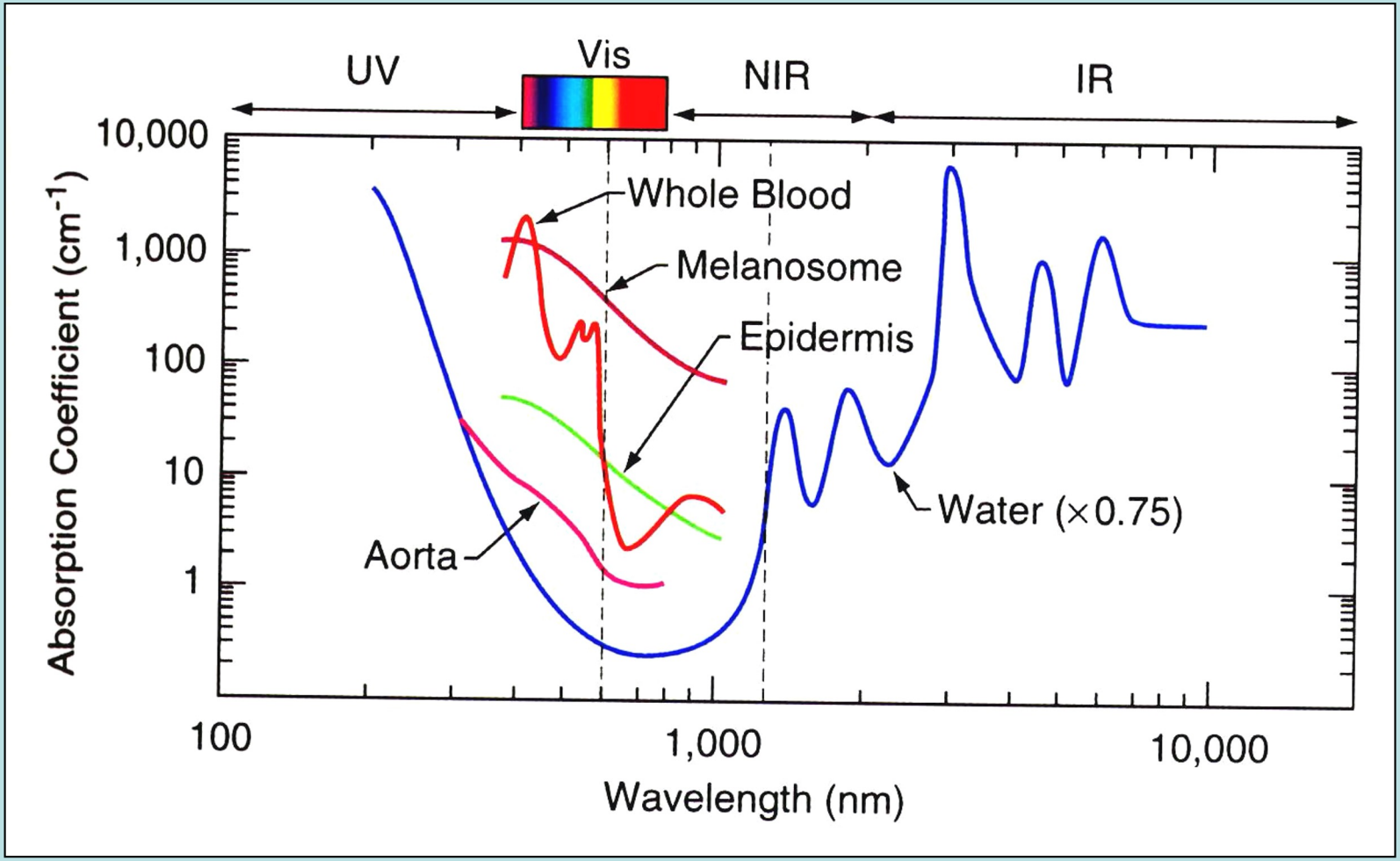
- ~ 5 mm at λ = 800 nm
- <1 μm at λ=2.94 μm
- ≤ 20 μm throughout $\lambda \geq 6$ μm

Skin spectral reflectance in the visible and IR

Summary - Absorber

UV	Visible & NIR	IR
• Protein • Amino acid • Fatty Acid • Peptide • DNA • NADH • FAD • Water	• Hemoglobin • Lipid • Cytochrome a3 "Therapeutic Window" 600 nm ~ 1000 nm	• Water • Protein • Glucose

Optical Absorption in Tissues

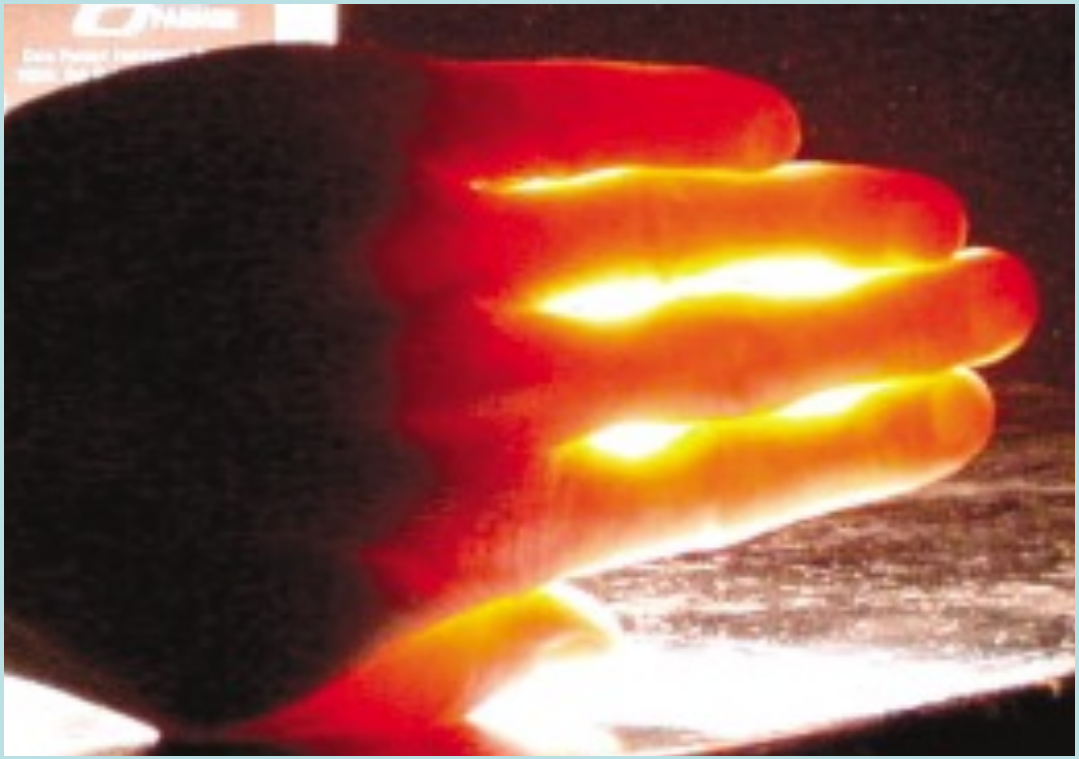


Ref: Vo-Dinh, Biomedical Photonics Handbook

SCATTERING in TISSUE

Light Propagation in Media (Tissues)

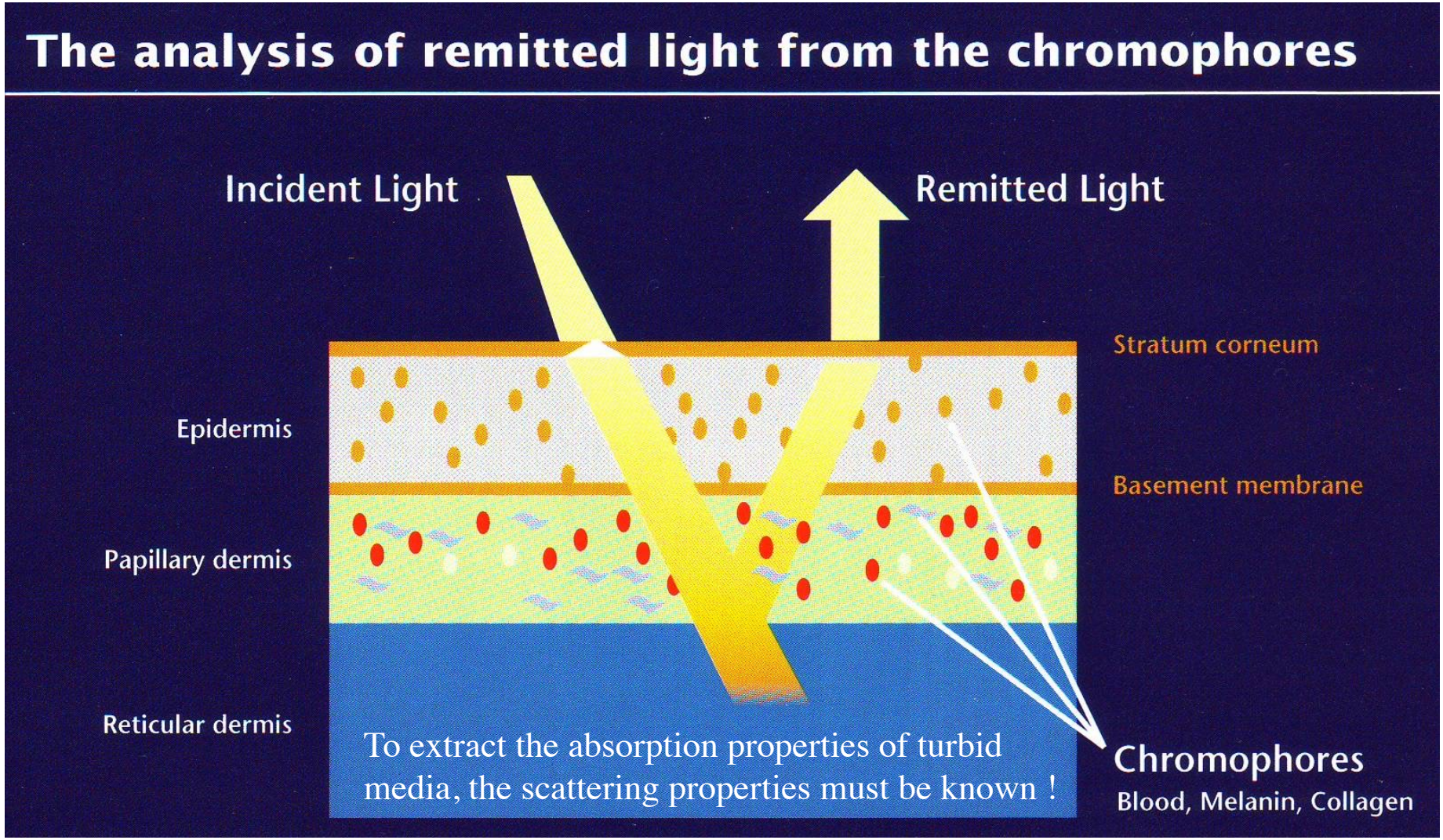
…includes Reflection, Refraction, Absorption and Scattering



Scattering in Tissue Optics

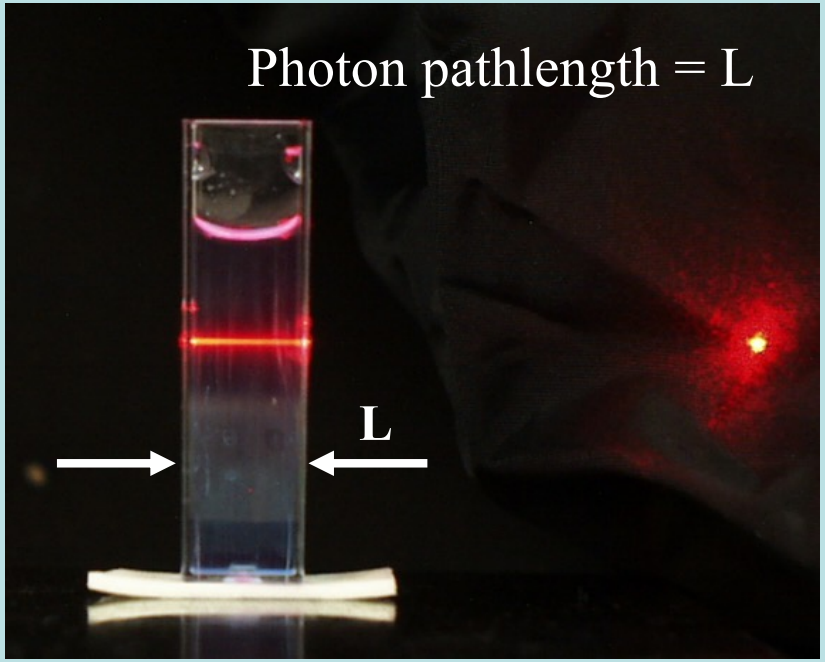
Mother of all confusion in tissue optics !!!

- Much more complicated than absorption
- Light is hardly observed from the source, but reaches our eyes indirectly through scattering
- Inhomogeneity causes scattering: cloud, raindrop, etc.
- Elastic (Rayleigh, Mie) or inelastic (Raman)

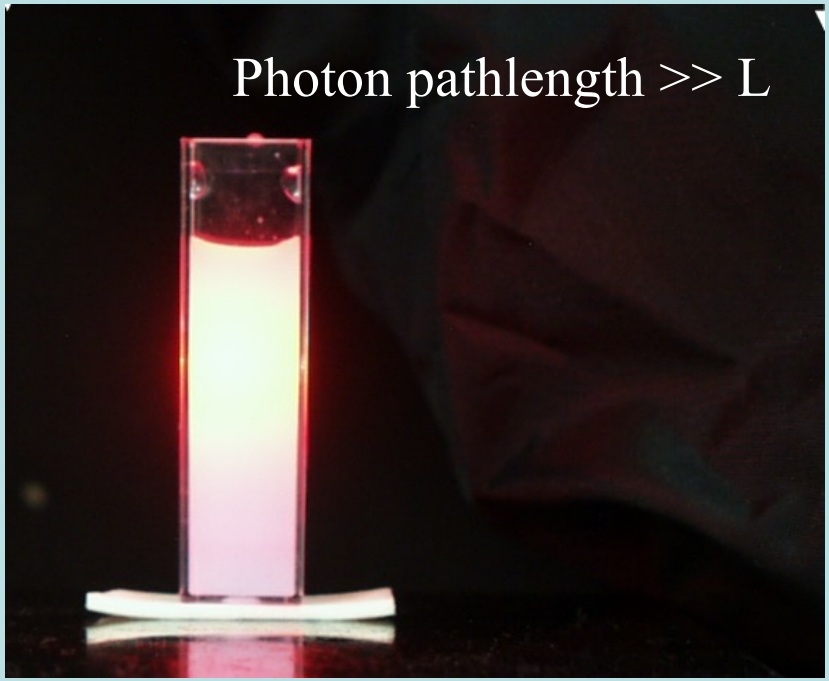

The analysis of remitted light from the chromophores
Incident Light
Remitted Light
Stratum corneum
Epidermis
Basement membrane
Papillary dermis
Reticular dermis
To extract the absorption properties of turbid media, the scattering properties must be known !
Chromophores
Blood, Melanin, Collagen

Scattering - Example

"Purely" absorbing



With Scattering



Lambert- Beer Law does not apply here!!!
Need to calculate true path length of light

Some Definitions

Scattering = light is forced to deviate from a straight trajectory by one or more localized non-uniformities in the medium through which it passes.
key word: deviation by a non-uniformities

Diffusion = photons travelling through a material with a high optical depth and very short mean free path. Their behaviour is then dominated by scattering and the path of any given photon is effectively a random walk.
key word: random motion

Diffraction = Various phenomena associated with light propagation, such as the bending, spreading and interference of waves passing by an object or aperture that disrupts the wave.

Scattering

= Deviation of light from a straight trajectory by non-uniformities in the tissue (depends on the size, morphology, and structure of the components in tissues (e.g. lipid membrane, collagen fibers, nuclei)).

Diagnostic applications

Variations in the scatterers due to disease would affect scattering properties, thus providing a mean for diagnostic purpose

Therapeutic applications

Scattering signals can be used to determine optimal light dosimetry and provide useful feedback during therapy

- Many spectroscopic techniques are based on scattering
  (Laser Doppler Perfusion, Optical Coherence Tomography, Raman Scattering Spectroscopy)
- To be able to extract the absorption properties of turbid media, the scattering properties must be known in certain cases

Mechanism for Light Scattering

Light scattering arises from the presence of heterogeneities within a bulk medium

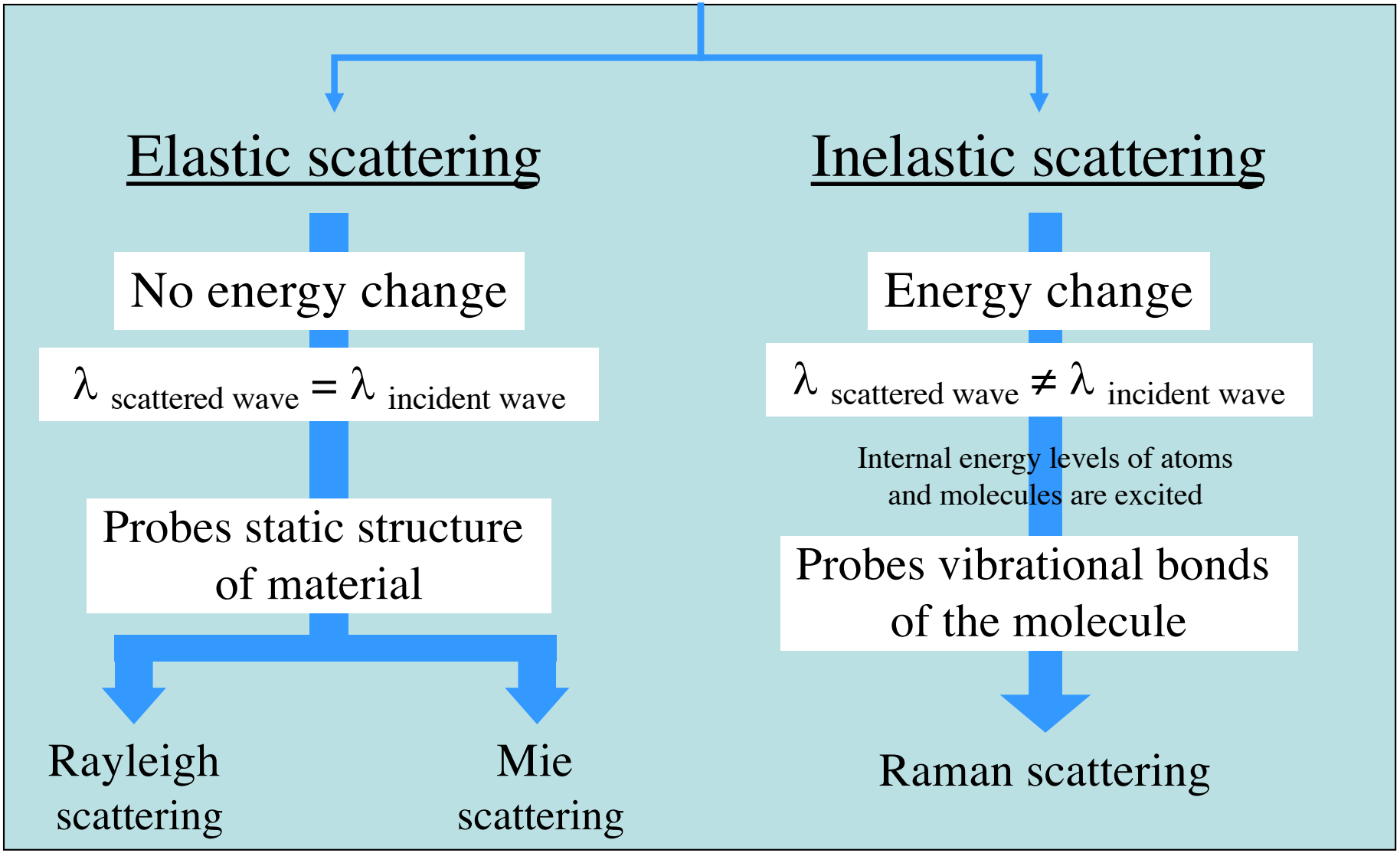
Heterogeneities result in non-uniform temporal/spatial distribution of refractive index in the medium

Passage of an incident EM wave sets electric charges into oscillatory motion and can excite vibrational modes

Scattered light is re-radiated by acceleration of these charges and/or relaxation of vibrational transition

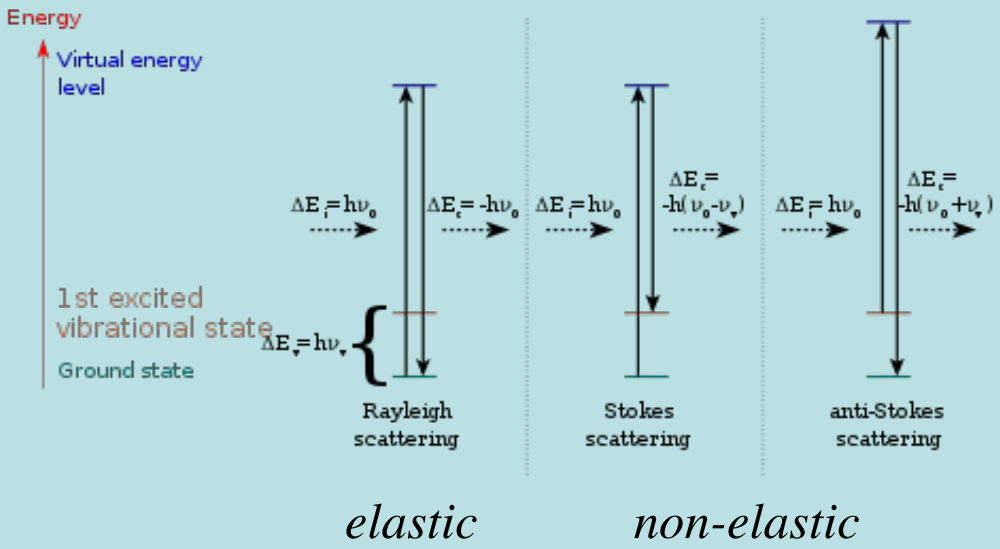
- Physical inclusions
- Fluctuations in dielectric constant from random thermal motion

Scattering


Elastic scattering
No energy change
λ scattered wave = λ incident wave
Probes static structure of material
Rayleigh scattering
Mie scattering
Inelastic scattering
Energy change
λ scattered wave ≠ λ incident wave
Internal energy levels of atoms and molecules are excited
Probes vibrational bonds of the molecule
Raman scattering

Mechanism of Raman scattering

The energy diagram of a molecule showing the origin of Raman scattering



elastic *non-elastic*

- Inelastic scattering
- Photon is considered to undergo absorption and subsequent emission via an virtual electron state
  (virtual means that the molecule is momentarily elevated to a higher energy level but it never reaches an electronic excited state.)

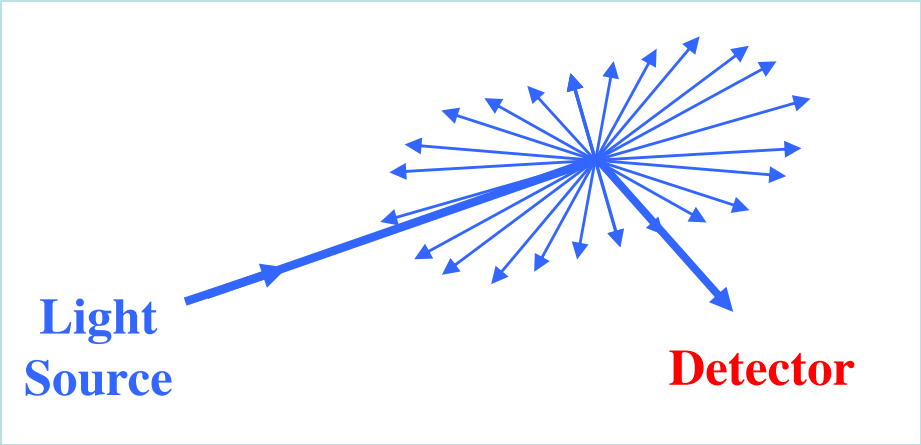
scattered photons have energies $E = h(\nu_0 \pm \nu_v)$
+:Stokes line; -: Anti-Stokes line

Elastic Scattering

- Photons are mostly scattered by the structure whose size matches the wavelength

- Principal parameters that affect scattering
  - Wavelength
  - Relative refractive index
  - Particle radius
  - Shape and orientation

Rayleigh Scattering



$$I = I_0 \frac{8\pi^4 N\alpha^2}{\lambda^4 R^2}(1+\cos^2\theta)$$

N = number of scatterers
α = polarizability
R = distance from scatterer

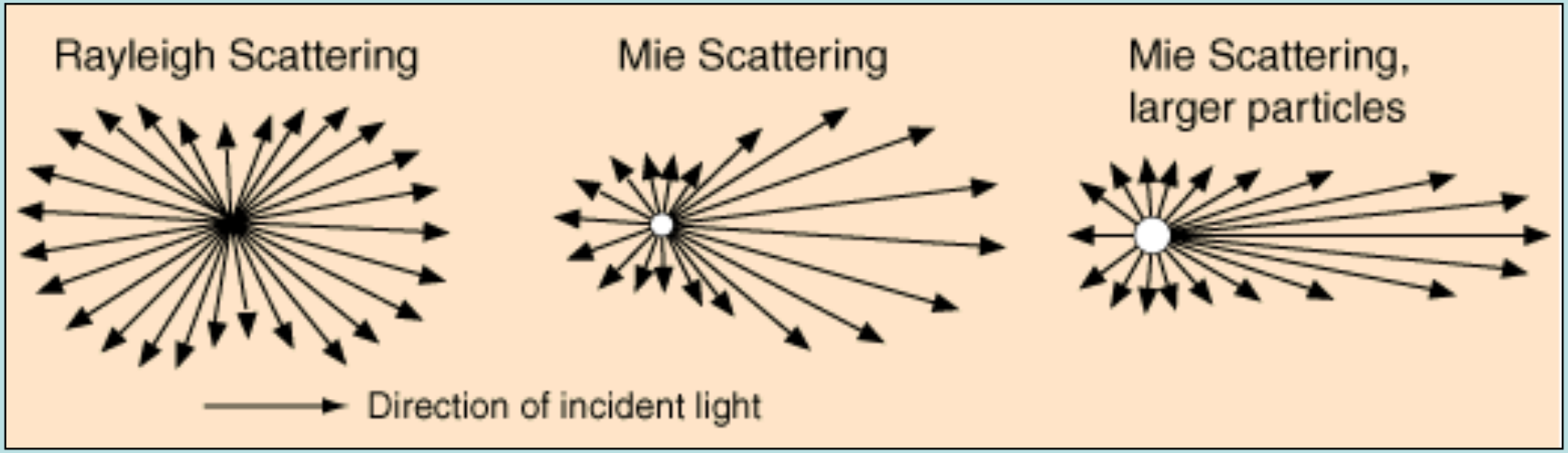
Properties of Rayleigh scattering:

- Scattering from very small particles → ≤ λ/10
- Scattering at right angles is half the forward intensity
- The strong wavelength dependence enhances the short wavelengths
  (Rayleigh scattering is inversely related to the fourth power of the wavelength of the incident light)

$$I \propto \frac{1}{\lambda^4}$$

Mie Scattering

- Scattering of particles comparable or larger than the wavelength, Mie scattering predominates
- Because of the relative particle size, Mie scattering is poorly wavelength dependent
- Forward directional scattering

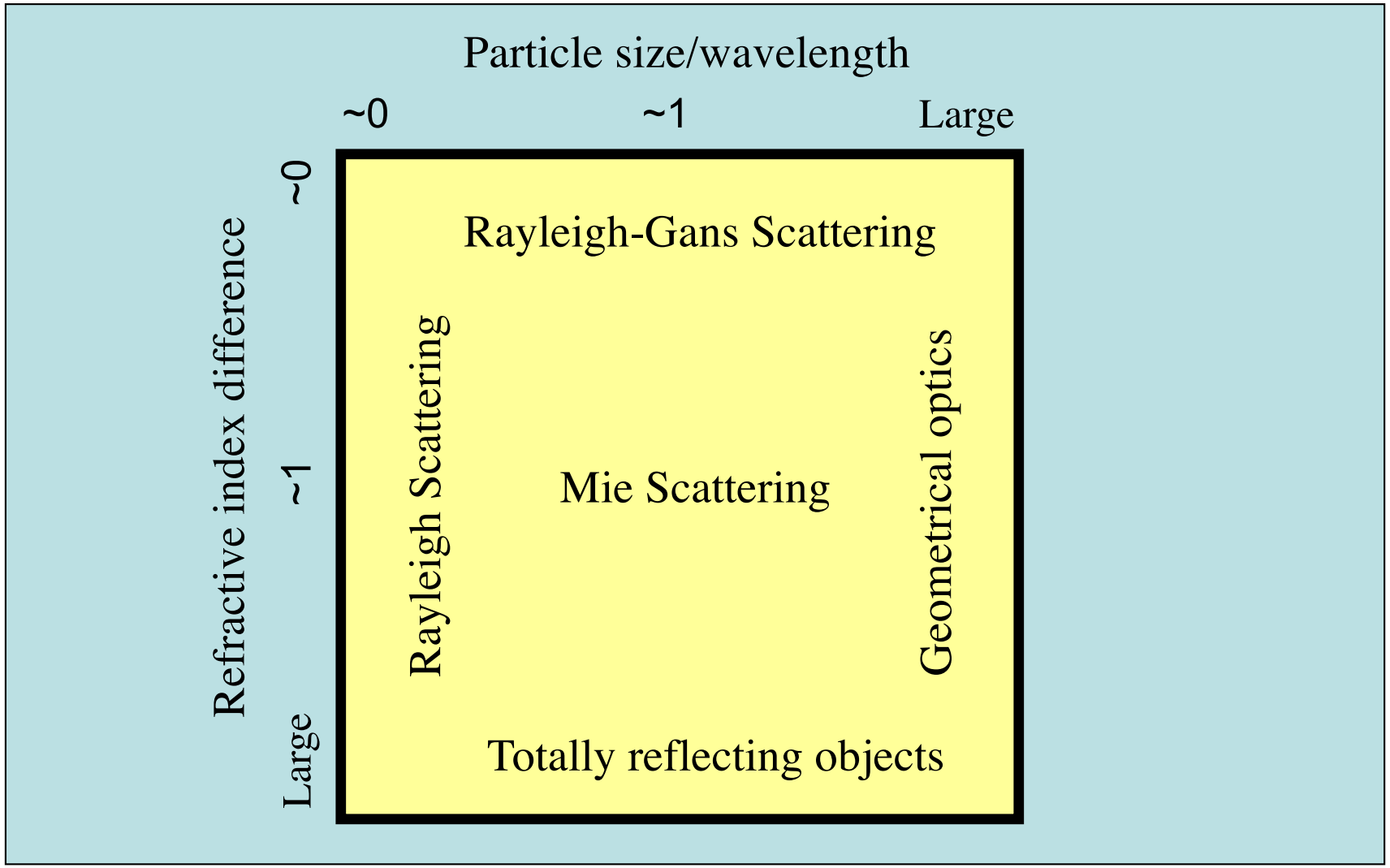


Scattering – Blue sky revisited

- Blue skies are produced due to scattering at shorter wavelengths
- Visible light (violet & blue) are selectively scattered by O_2 and N_2 – much smaller than wavelengths of the light
- Violet and blue light has been scattered over and over again

- Mie scattering is poorly wavelength dependent – appears white
- When light encounters larger particles (cloud, fog,cigarette smoke), Mie scattering occurs

Electromagnetic Scattering

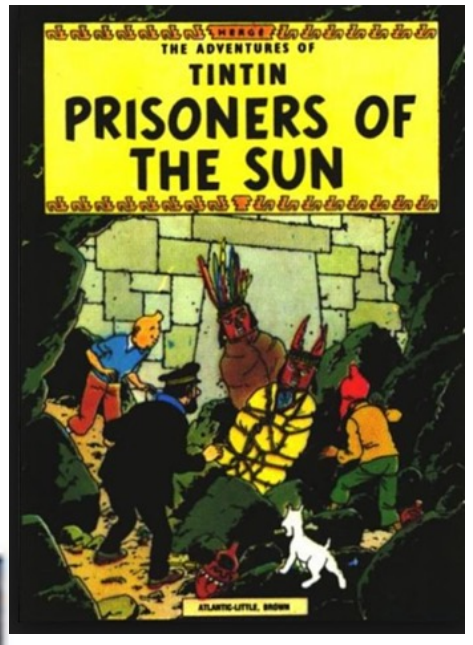


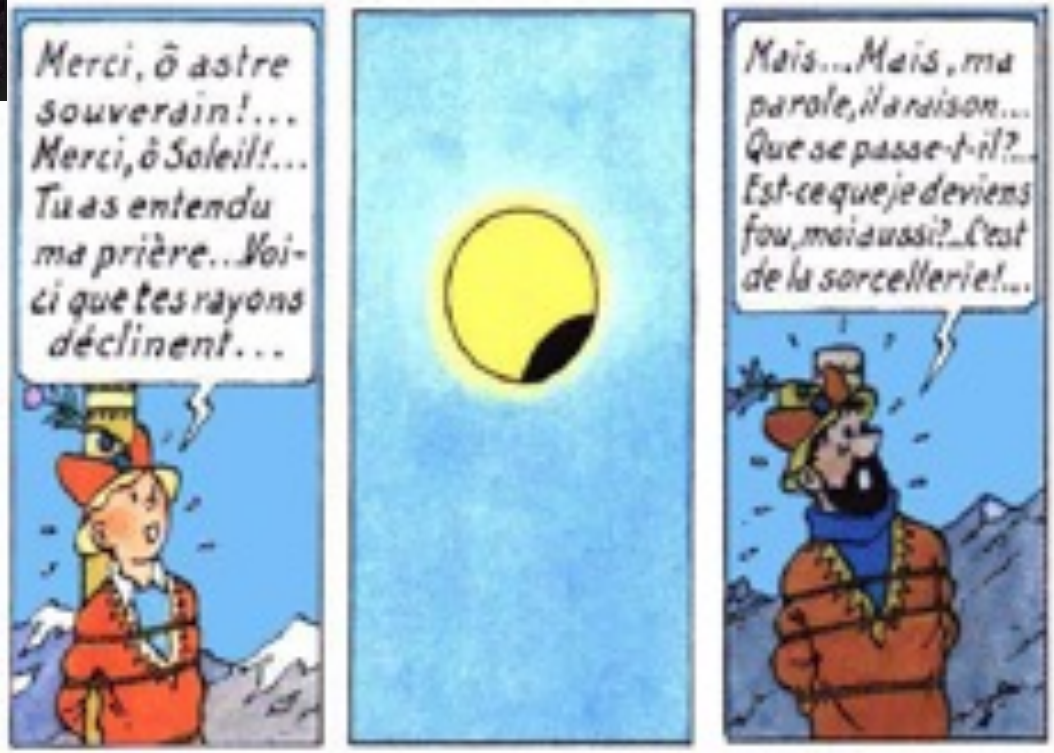
"General" dependences of Scattering

- Scattering decreases monotonically with increasing wavelength
- Scattering coefficient increases with increasing diameter of the scatterer
- Scattering coefficient increases with refractive index mismatch

Machu Picchu

Machu Picchu





Machu Picchu

Machu Picchu

Nice weather

Bad weather

Scattering of the atmosphere

Metrics for Optical Scattering

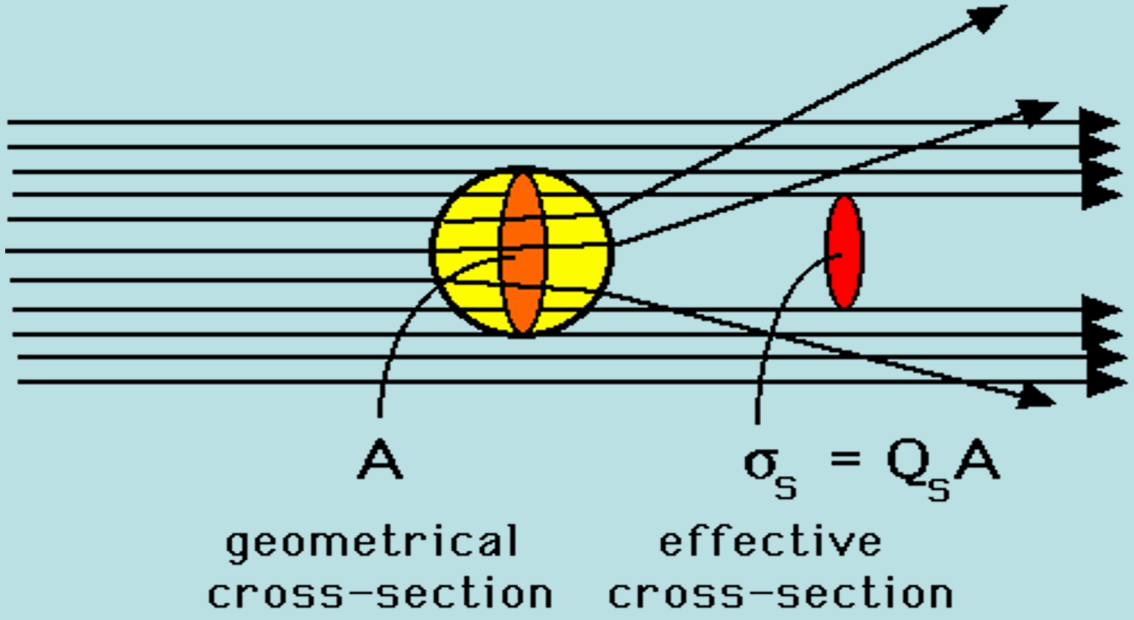
Scattering Cross Section σ_s [cm^2]

for a single scatterer

$$\sigma_s = Q_s \cdot A_s$$

Q_s = scattering efficiency (can be calculated from Mie theory)

A_s = the area of the scatterer (cm^2)



Metrics for Optical Scattering

Scattering Coefficient μ_s [m^{-1}]

- Cross-sectional area for scattering per unit volume of medium

$$\mu_s = N_s \sigma_s$$

N_s = the number density of scatterers

σ_s = scattering cross-section

Scattering Mean Free Path l_s [m]

- Average distance a photon travels between scattering events

$$l_s = \frac{1}{\mu_s}$$

Note!
l_s is sometimes also written as
mfp = mean free path

Direction of Scattering

	Mie Scattering	Rayleigh scattering
Wavelength dependence	$\sim \lambda^{-x}$, x is "small" (0.5)	$\sim \lambda^{-4}$
Direction	preferably forward	forward and backward (proportional to $[1+\cos^2(\theta)]$)

Biological tissue:

- preferably forward directed scattering
- wavelength dependence stronger than for Mie scattering

Neither Rayleigh nor Mie theory completely describe scattering in biological tissue!

Define probability function $p(\theta)$ of a photon to be scattered

Scattering Phase Function

Differential scattering cross section:

scattering in direction s' from input direction s

$$\frac{d\sigma_s}{d\Omega}(\hat{s},\hat{s}')$$

- *The probability to observe a scattered particle in a given state per solid angle unit if the target is irradiated by a flux of one particle per surface unit*

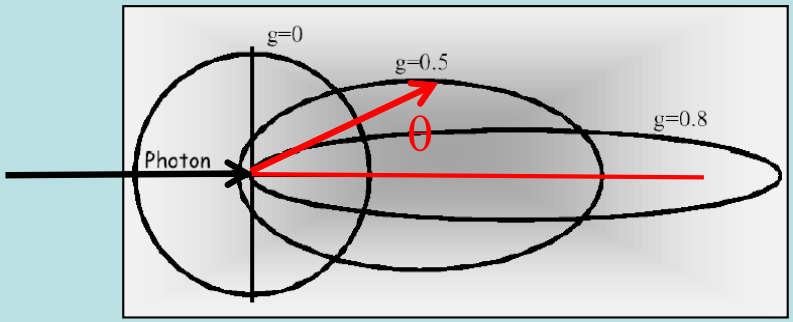
Often only depends on angle between input and output then:

- The angular dependence of scattering is called the scattering probability function p(θ)

Scattering Phase Function

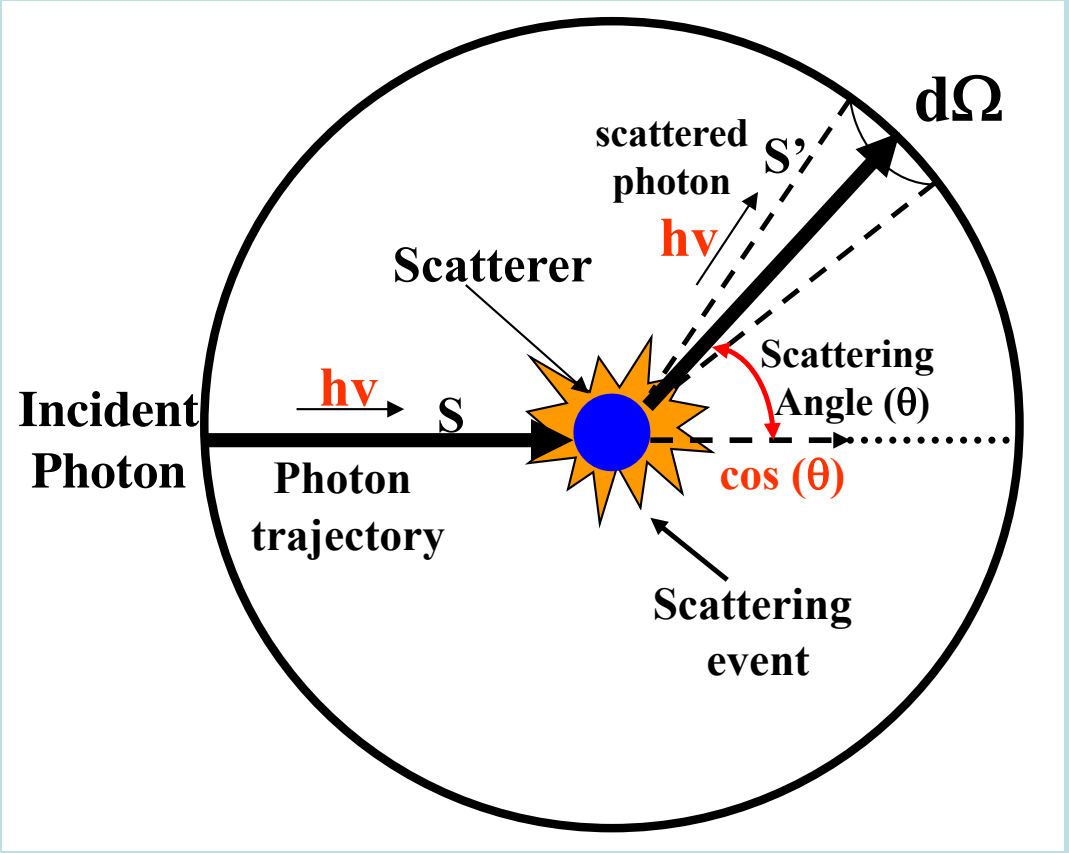
p(θ) often only depends on angle between input and output!

- *p(θ) describes the probability of a photon to be scattered into a unit solid angle, relative to the original photon trajectory*
- The angular dependence of scattering p(θ) has historically been called the **scattering phase function**



Scattering Anisotropy g

- Imagine that a photon is scattered by a particle so that its trajectory is deflected by an angle θ
- Then, the component of the new trajectory along the forward direction is cos(θ)



Average cos(θ):

Forward scattering	0...1
Backward scattering	-1... 0

The anisotropy $g = \langle \cos(\theta) \rangle$

is a measure of the forward direction retained after a single scattering event

Scattering Anisotropy

The proper definition of anisotropy (g) is the expectation value for cos (θ):

"Effectiveness of Scattering"

$$g \equiv \frac{\int p(\hat{s}\cdot\hat{s}')\,\hat{s}\cdot\hat{s}'\,d\Omega}{\int p(\hat{s}\cdot\hat{s}')\,d\Omega}$$

If p only depends on the angle between input and output

$$g = <\cos\theta> = \int_0^{\pi} p(\theta)\cos\theta\cdot 2\pi\sin\theta\, d\theta$$

$$where, \int_0^{\pi} p(\theta)\cdot 2\pi\sin\theta\, d\theta = 1$$

Sometimes also written in terms of cos(θ):

$$= \int_{-1}^{1} p(\cos\theta)\cos\theta\, d(\cos\theta)$$

$$where, \int_{-1}^{1} p(\cos\theta)\, d(\cos\theta) = 1$$

Anisotropy factor g

$$g = \begin{cases} -1 & \text{totally backward scattering} \\ 0 & \text{isotropic scattering} \\ 1 & \text{totally forward scattering} \end{cases}$$

Biological Tissues, $0.65 < g < 0.95$

Isotropic Phase Function

Isotropic scattering: scattering of light at equal efficiency into all possible directions

The phase function is $$p(\theta) = \frac{1}{4\pi}$$

The phase function for isotropic scattering is independent of θ !

Heyney Greenstein phase function

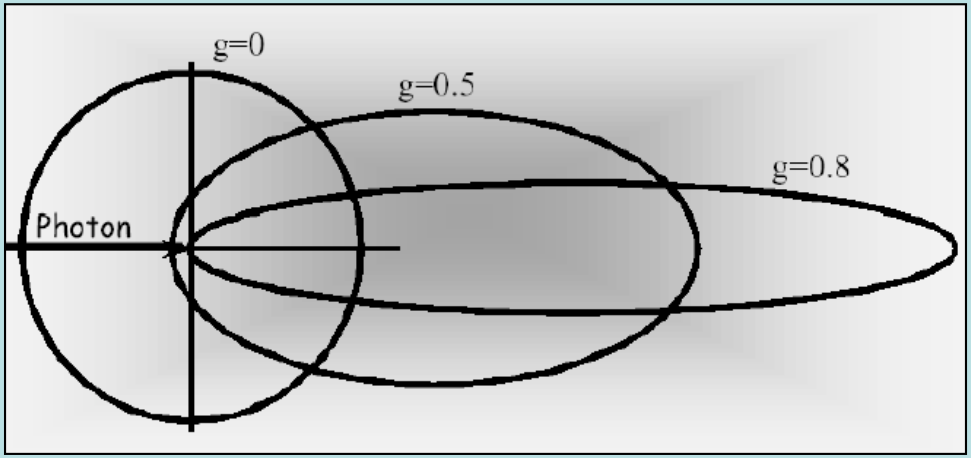
The Heyney Greenstein scattering phase function is an analytical expression which mimics the angular dependence of light scattering

$$p(\theta) = \frac{1}{4\pi} \frac{1-g^2}{\left(1+g^2-2g\cos\theta\right)^{3/2}}$$

where $\int_0^{\pi} p(\theta) 2\pi \sin\theta d\theta = 1$

and $\int_0^{\pi} p(\theta)\cos\theta 2\pi \sin\theta d\theta = g$

Probability distribution of scattered photon for g= 0, 0.5 and 0.8

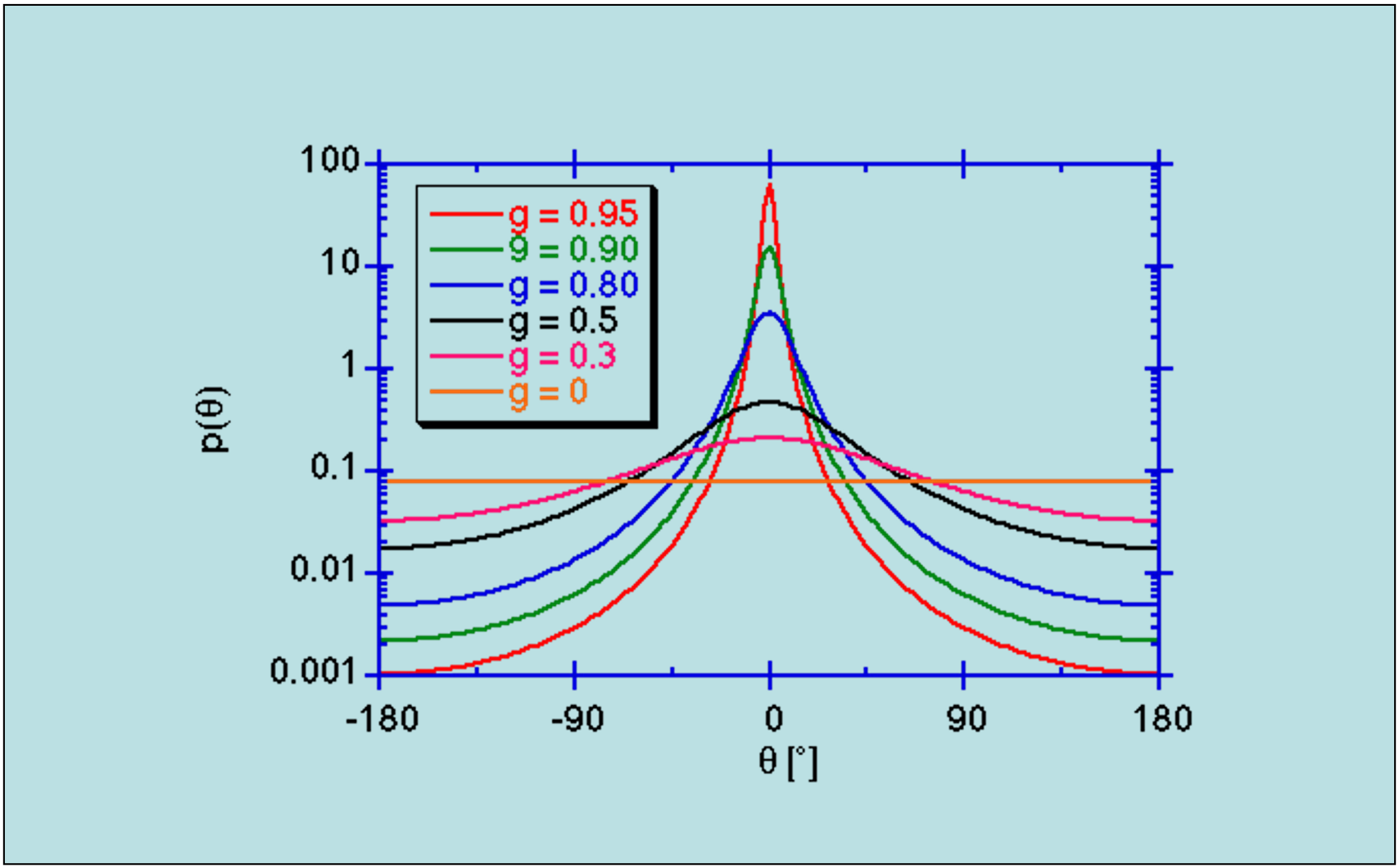


Henyey Greenstein Approximation

The function allows the anisotropy factor, g, to specify p(θ) such that $<cos(\theta)>$ returns to the same value of g.

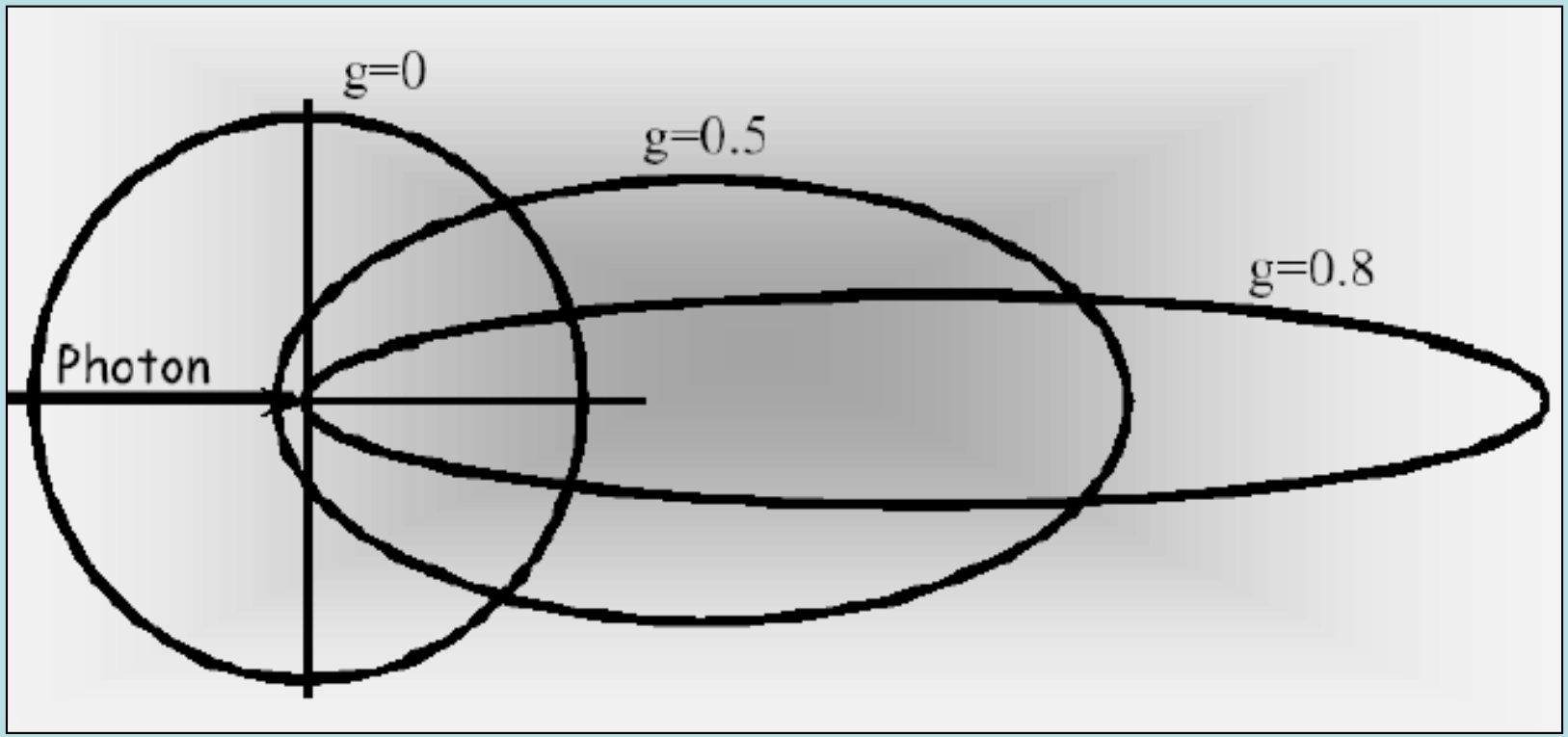
The function does not represent true scattering phase functions very well, but it is a good average approximation for biological tissues.

Example: Heyney Greenstein



Heyney Greenstein phase function

Probability distribution of scattered photon for g= 0, 0.5 and 0.8



Two solutions with identical macroscopic scattering properties can differ at a microscopic level!
μ_s and g are different but the macroscopic aspects are the same!

Metrics for Optical Scattering

Reduced Scattering Coefficient μ_s' [cm^{-1}]

- incorporates the scattering coefficient and the anisotropy factor

$$\mu_s' = \mu_s(1-g)$$

μ_s is the scattering coefficient [cm^{-1}]
g the anisotropy factor

- μ_s' can be regarded as an effective isotropic scattering coefficient that represents the cumulative effect of several forward-scattering events
- Special significant with respect to photon diffusion theory

Metrics for Optical Scattering

Reduced mean free path mfp' [m]

$$mfp' = \frac{1}{\mu_s'}$$

- purpose of the reduced scattering coefficient:
  to describe the diffusion of photons (**isotropic** scattering)
  in a random walk of step size ($1/\mu_s'$)

- Such a description is equivalent to the description of photon movement using many small mean free paths, $1/\mu_s$, that each involve only a partial deflection angle, θ (**anisotropic** scattering)

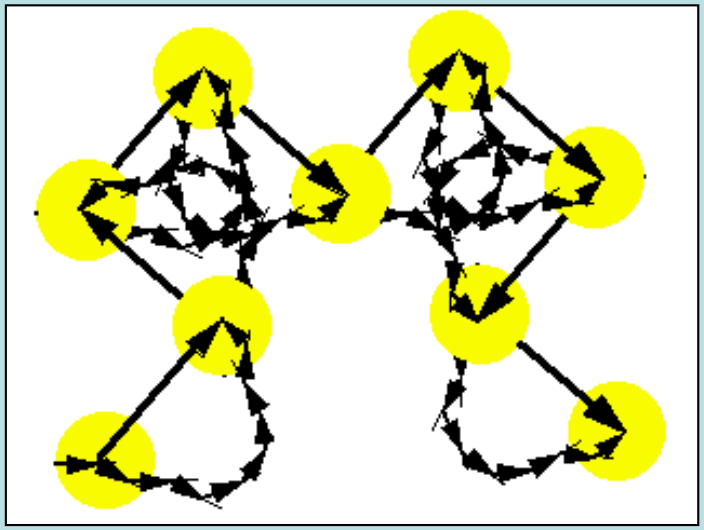
Reduced Scattering Coefficient

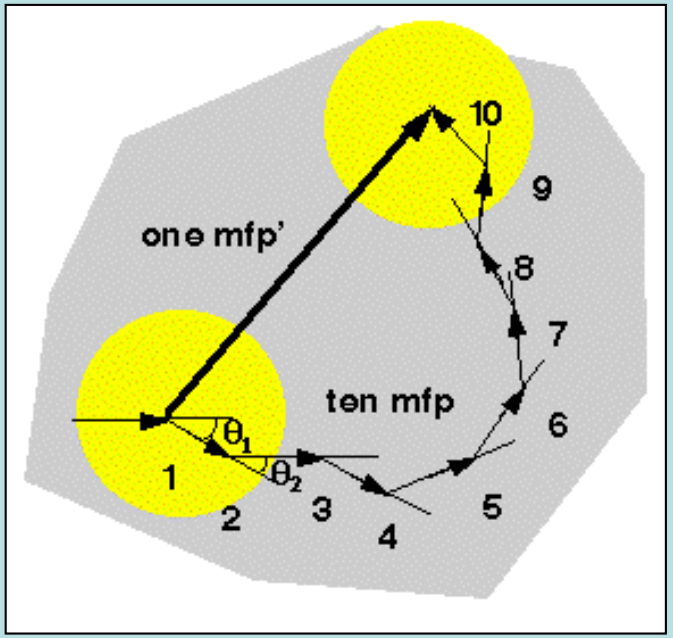
Useful for description of photon propagation in diffuse regime

Example: $g = \langle \cos\theta \rangle = 0.90 \Rightarrow \langle \theta \rangle \approx 26^{o}$

$$\mu_s' = (1-g)\mu_s = 0.10\mu_s$$

$$mfp = \frac{1}{\mu_s} \qquad mfp' = \frac{1}{\mu_s'}$$





Each step involves isotropic scattering.
Such a description is equivalent to description of photon movement using many small steps $1/\mu_s$ that each involve only a partial deflection angle

1 iso-scattering step = 1/(1-g) aniso-scattering. steps

Source of Scattering in Tissue

- Refractive index mismatch between lipid and surrounding aqueous medium
  - Light scattering in many soft tissues is dominated by lipid contents
  - Cellular membranes, membrane folds, and membraneous structure
- Mitochondria, ~ 1 μm
  - Intracellular organelle composed of many folded membrane
- Collagen fibers' diameter, 2 ~ 3 μm
  - Collegan fibrils, 0.3 μm

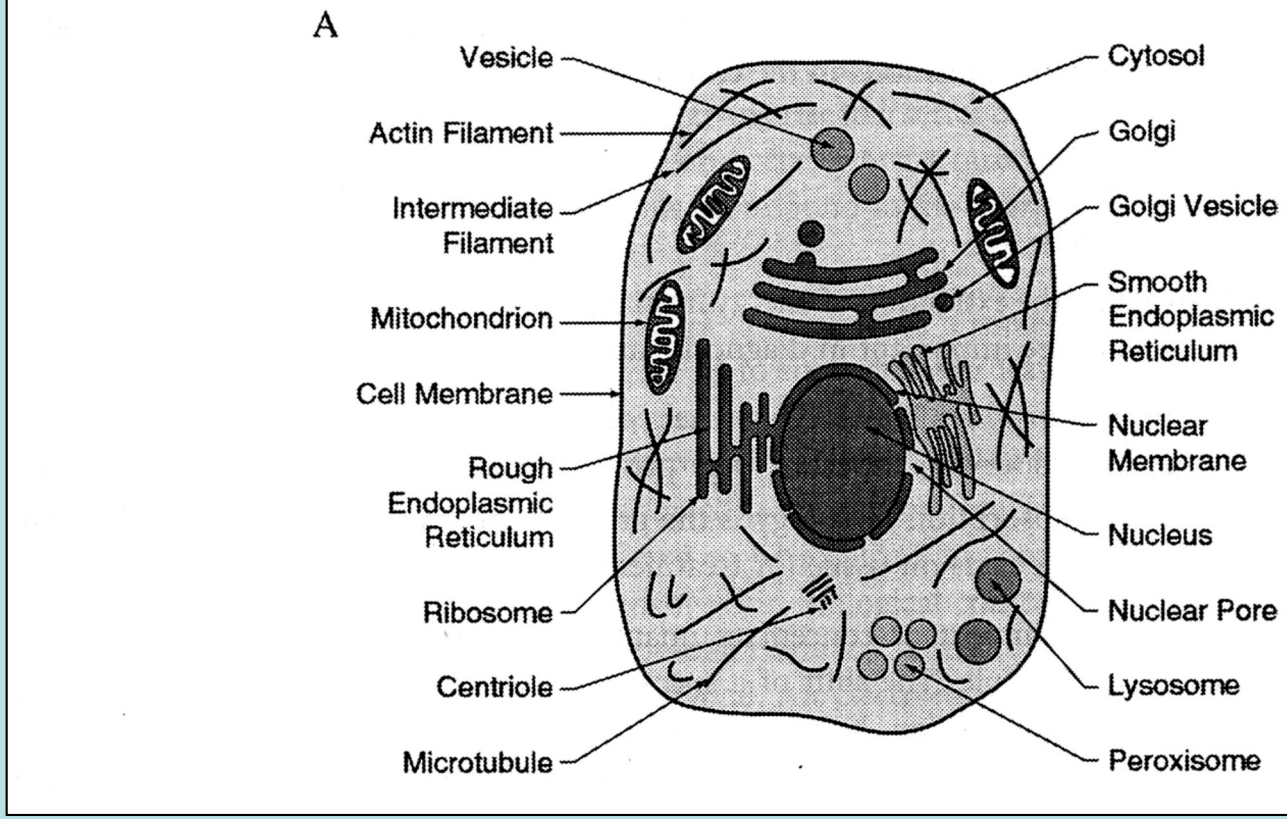
    (Periodic fluctuation in collagen ultrastructure)

    → source of Rayleigh scattering in UV and Visible range
- Cells

Biological scatterers in skin

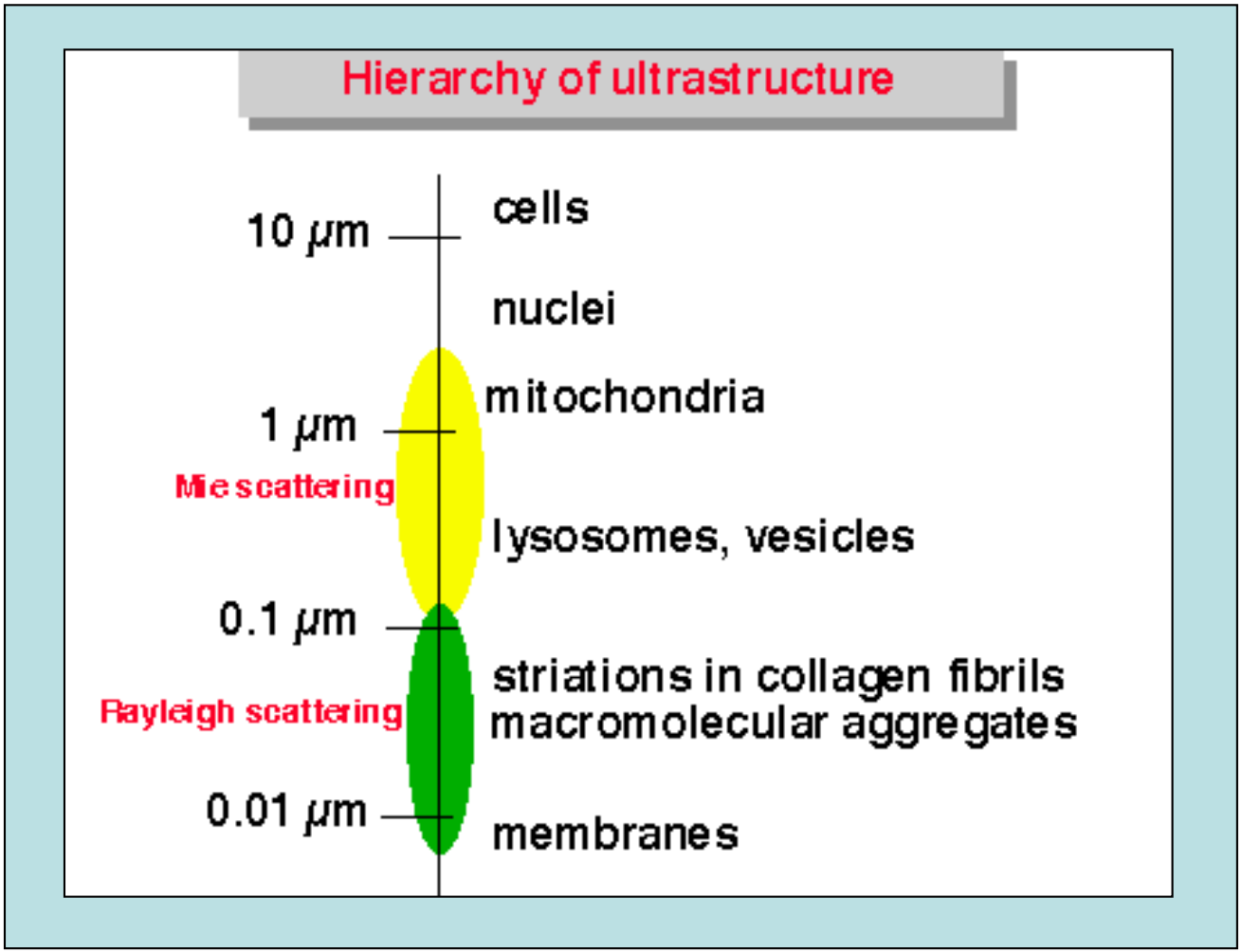
- Stratum corneum
  - Flat cells 0,6 - 0,8 μm thick
  - Visible range scattering.
- Epidermis
  - Melanin granules: 0,3 - 0,8 μm
  - Visible range scattering
- Dermis
  - Collagen fibrils: 20 - 100 nm
  - Rayleigh scattering
- Blood
  - Erythrocytes (7 - 8 μm);
  - leucocytes (9 - 40 μm);
  - platelets (1 - 3 μm)
  - Strong anisotropic scattering $g > 0,95$; $\mu_S > 50$ mm $^{-1}$

Sources of Scattering in Cells and Tissues

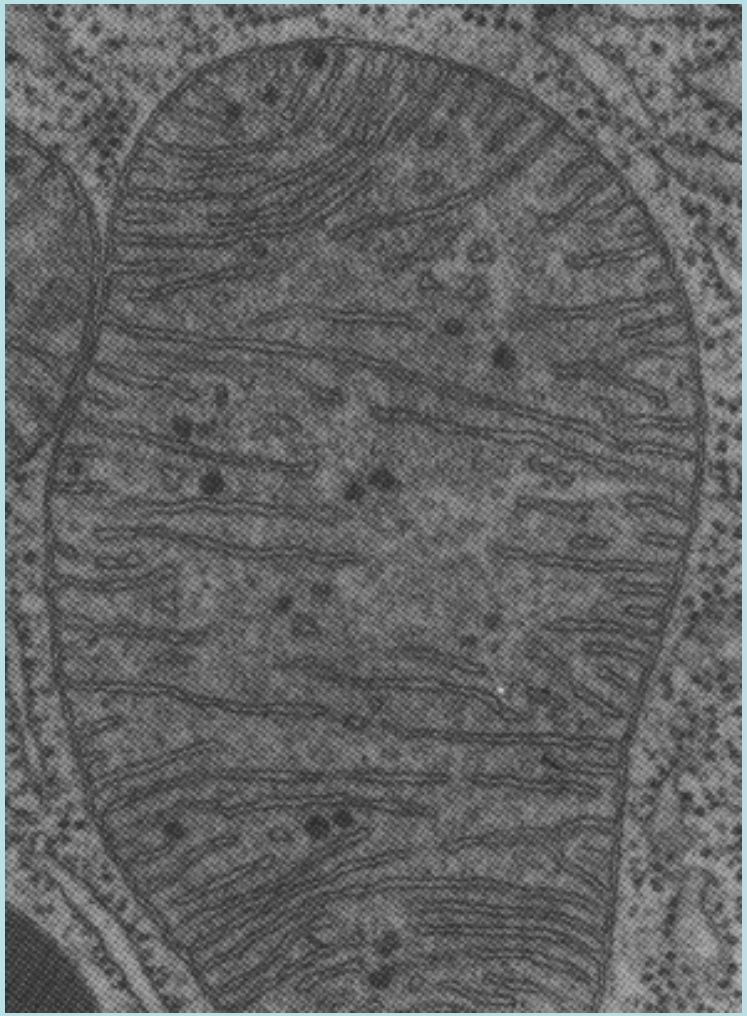


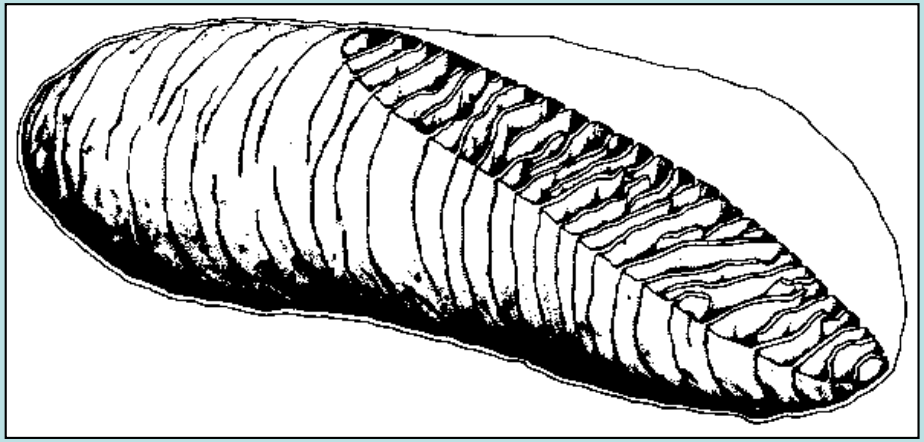
Ref: Vo-Dinh, Biomedical Photonics Handbook

Elastic Scattering: Biological Scatterers



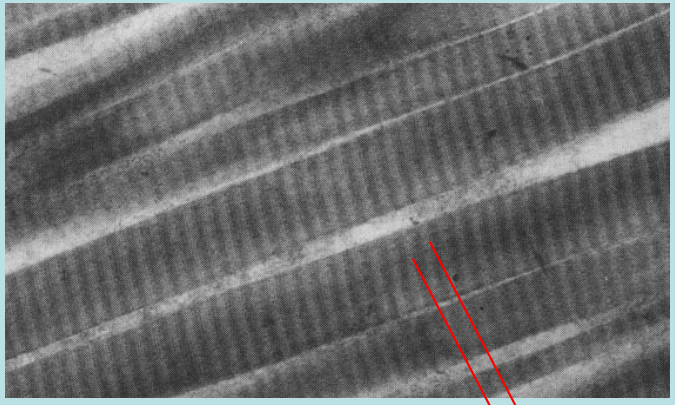
Mitochondria





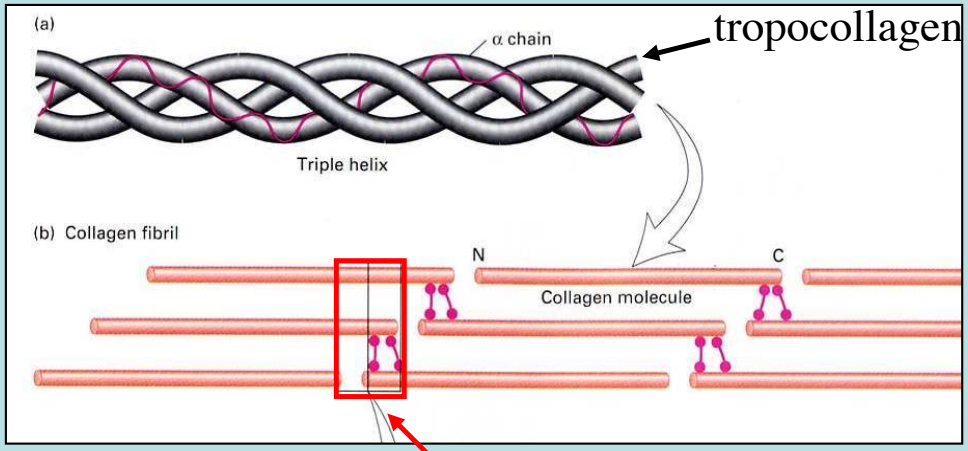
- 1 μm in size, folded lipid membranes, membranes 9 nm thick
- contains metabolic cofactors NAD, FAD used for proton pump over membrane to generate ATP
- Refractive index mismatch between lipid and water causes scattering

Collagen fibers and fibrils



(electron micrograph)

67 nm



Fibers: 2-3 μm in diameter;
composed of smaller fibrils (d = 4 nm; l = 300 nm)

→ strong Mie scattering

Fibrils: composed of tropocollagen molecules, have banded pattern (67 nm period), optical "crystal" 2nd harmonic generators, periodic structure contributes to...

→ Rayleigh scattering (visible and UV range)

- Cross Links, hydroxylysine pyridinoline and lysyl perydinoline are fluorescent

Biological Example: Soft Tissue (1)

- Scattering in most soft tissues is dominated by lipid content
- The lipids constitute the cellular membranes, membrane folds and membranous structures
- The lipid / water interface of membranes presents a strong refractive index mismatch and so plays a major role in scattering

Lipid membrane of cells:	n = 1.46
Cytosol of cells:	n = 1.35

Biological Example: Soft Tissue (2)

Selection of parameters

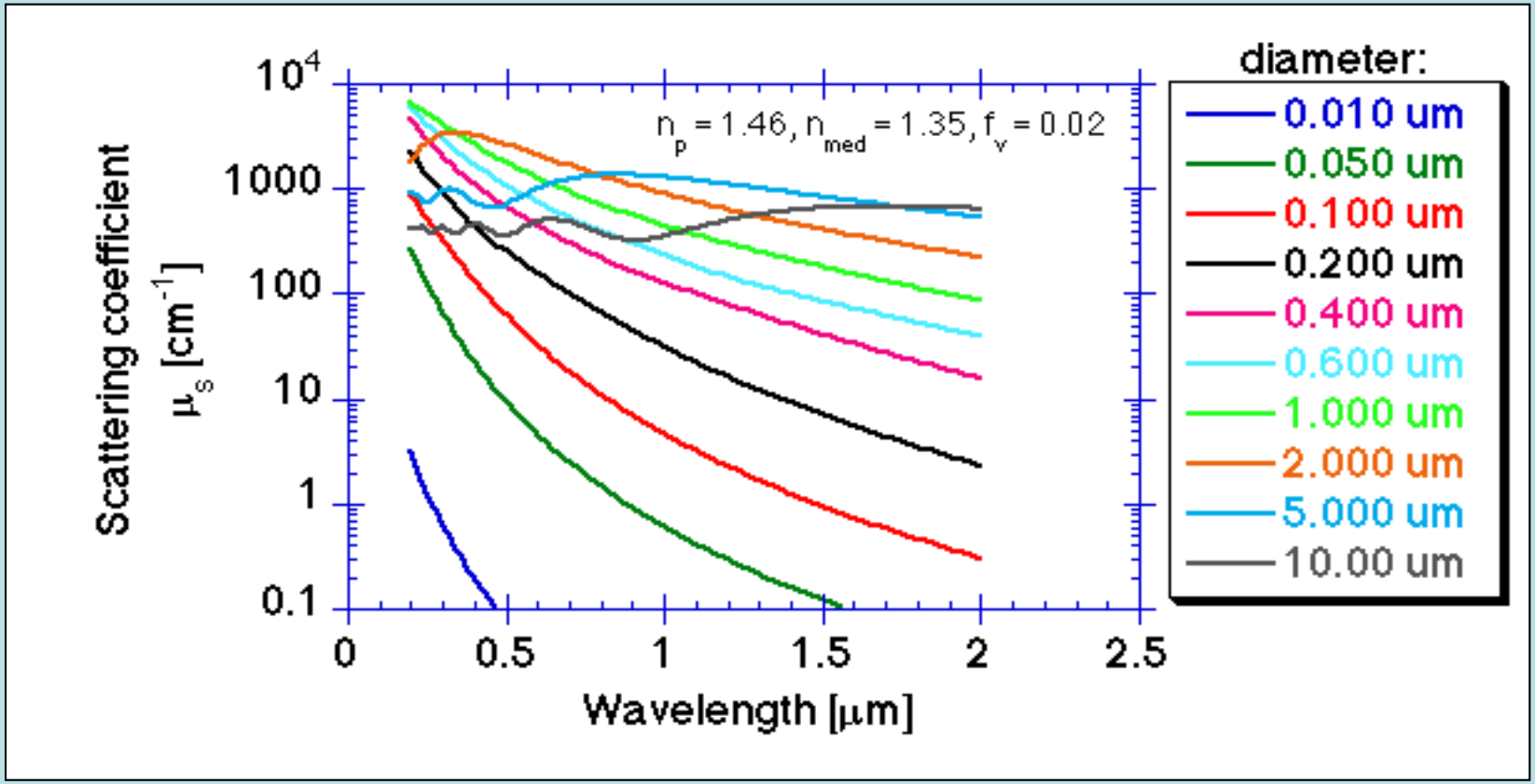
- Assume the lipid is packaged as small spheres of various sizes whose number density maintains a constant volume fraction, f_v (the proportion of the volume occupied by the scatterers's matter).
  Assume lipid content is $f_v = 2\%$
- The volume density (the number of spheres per volume unit), ρ_s is as follows (where a is the radius):

$$\rho_s = \frac{f_v}{(4/3)\pi a^3}$$

Biological Example: Soft Tissue (3)

Scattering coefficient computed for the soft tissue model for different diameters of "lipid spheres"

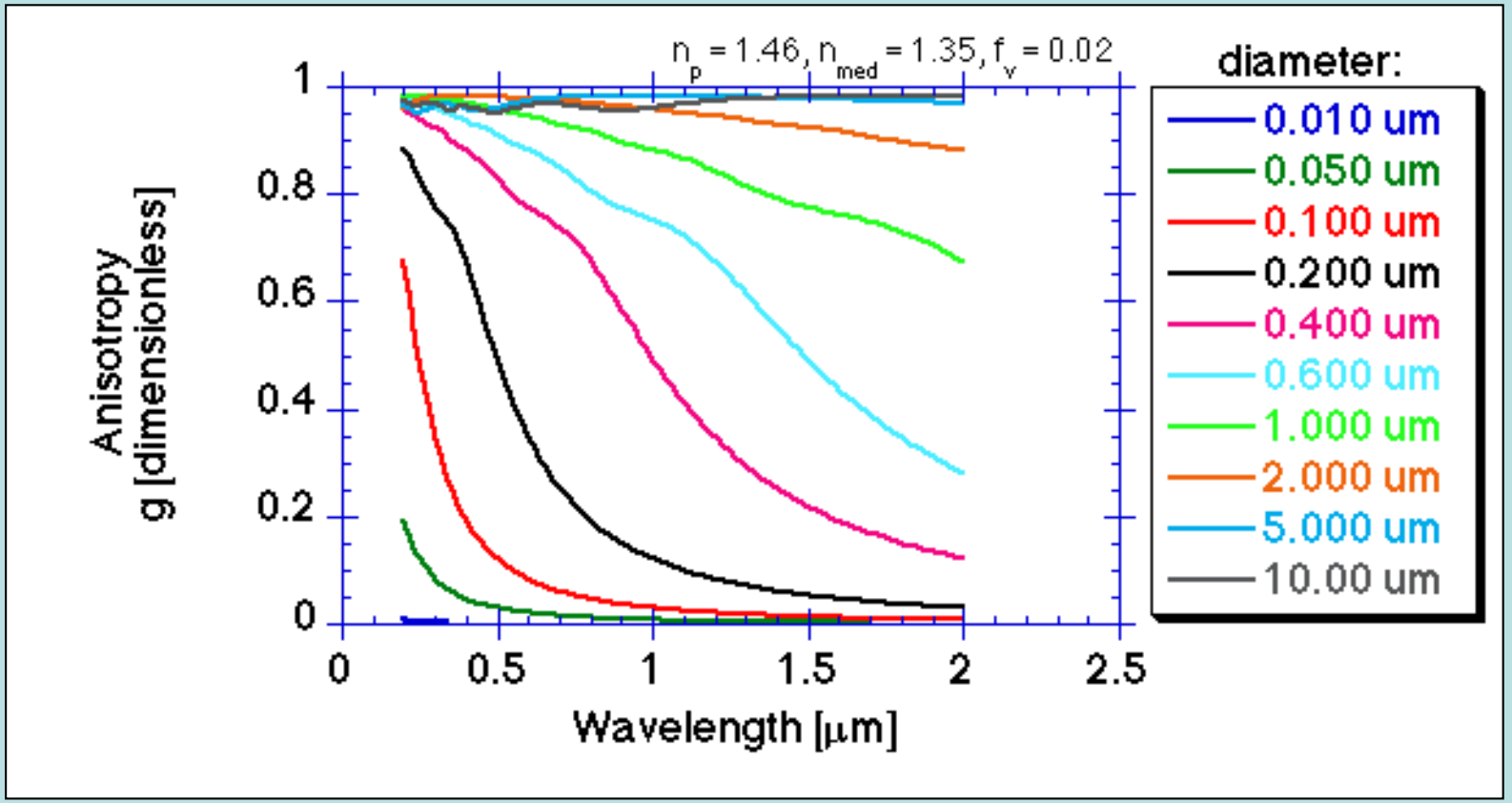
n_p= refraction index lipid (protein), n_{med}= refraction index cell (medium)



Biological Example: Soft Tissue (4)

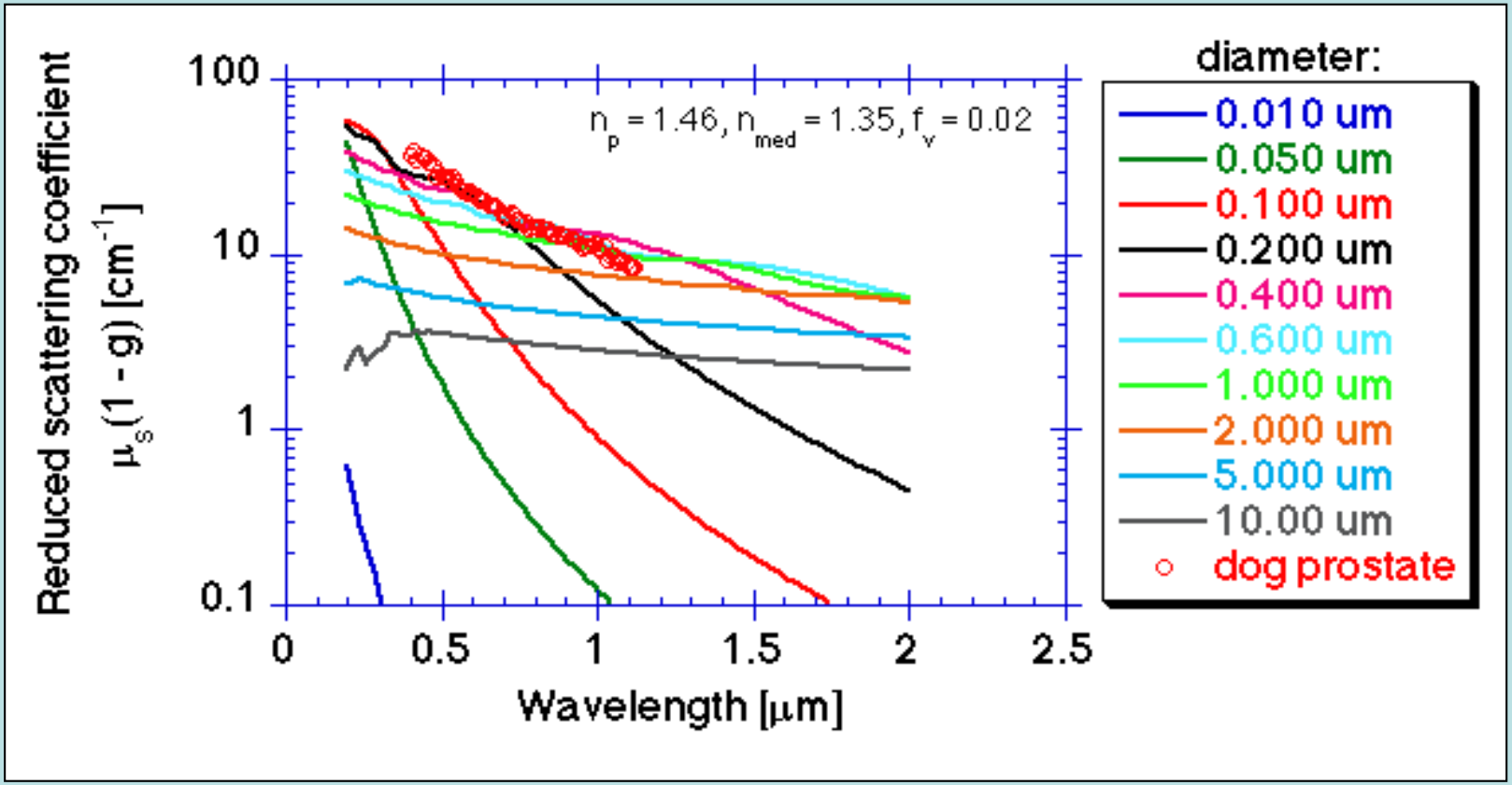
Anisotropy factor g for different diameters of "lipid spheres"

g = 0 ➡ isotropic; g = 1 ➡ forward peaked.



Biological Example: Soft Tissue (5)

Reduced Scattering coefficient for different diameters of lipid spheres in the range of 200 - 600 nm



Biological Example: Skin/ Collagen (1)

Skin (parameters drawn from the literature)

- Dermis of Skin and sclera of eye are highly scattering and have high collagen content
- **Macroscopic Level**: banded structure between tropocollagen fibrils. 0.3 micron fibrils – 8 micron fibers
- Fiber diameter: 2.8 +/- 0.08 micron
- Fiber Concentration / Volume Density:

$$\rho_s = 3 \times 10^6 \pm 0.5 \times 10^6 \text{ cm}^{-3}$$

- Volume fraction (surface density):

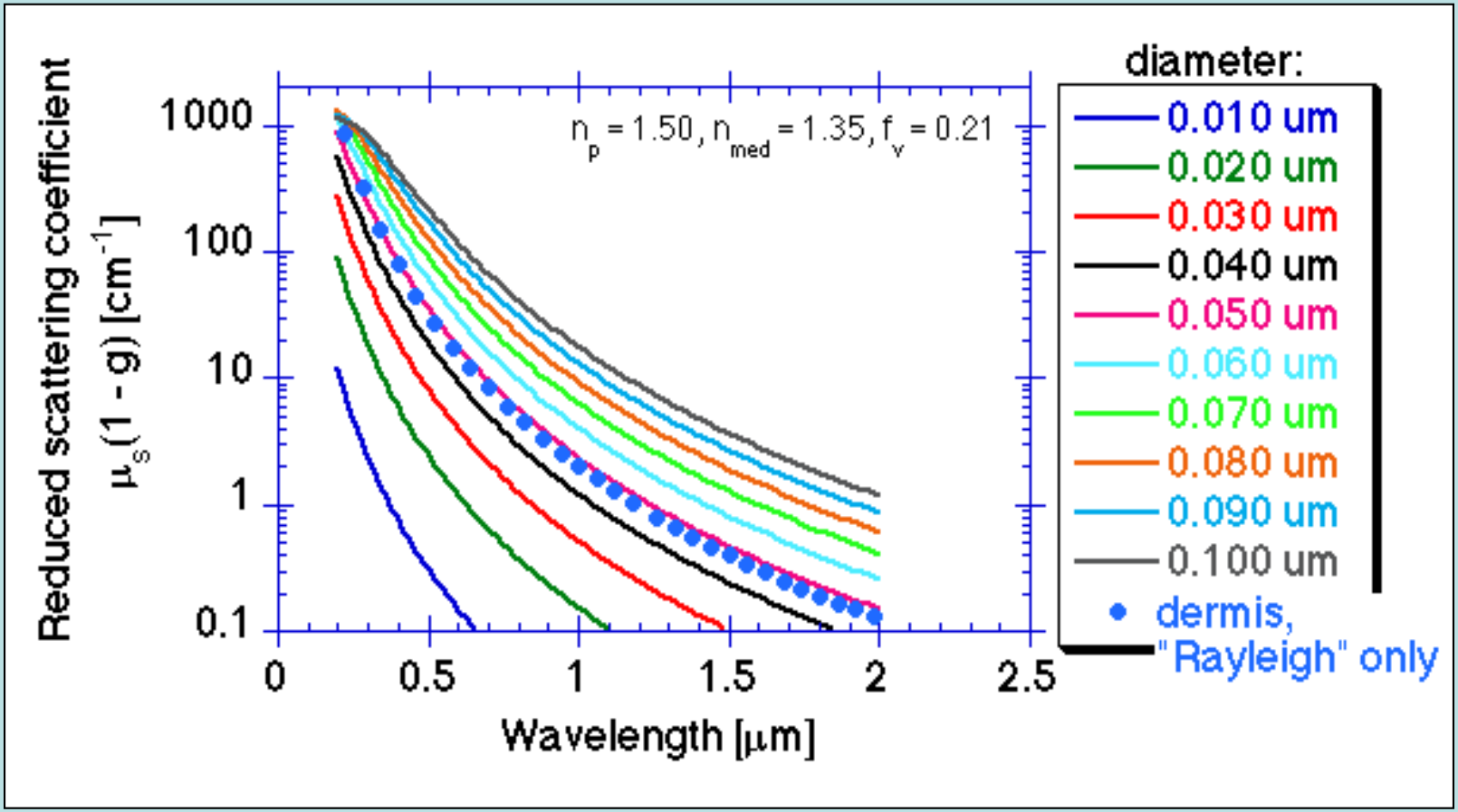
$$f_v = \pi a^2 [1cm] \rho_s = 0.21 \pm 0.1$$

n_{med}= 1.35 (dermal ground substrate),

n_{fiber}= 1.5 (collagen)

Biological Example: Skin/ Collagen (2)

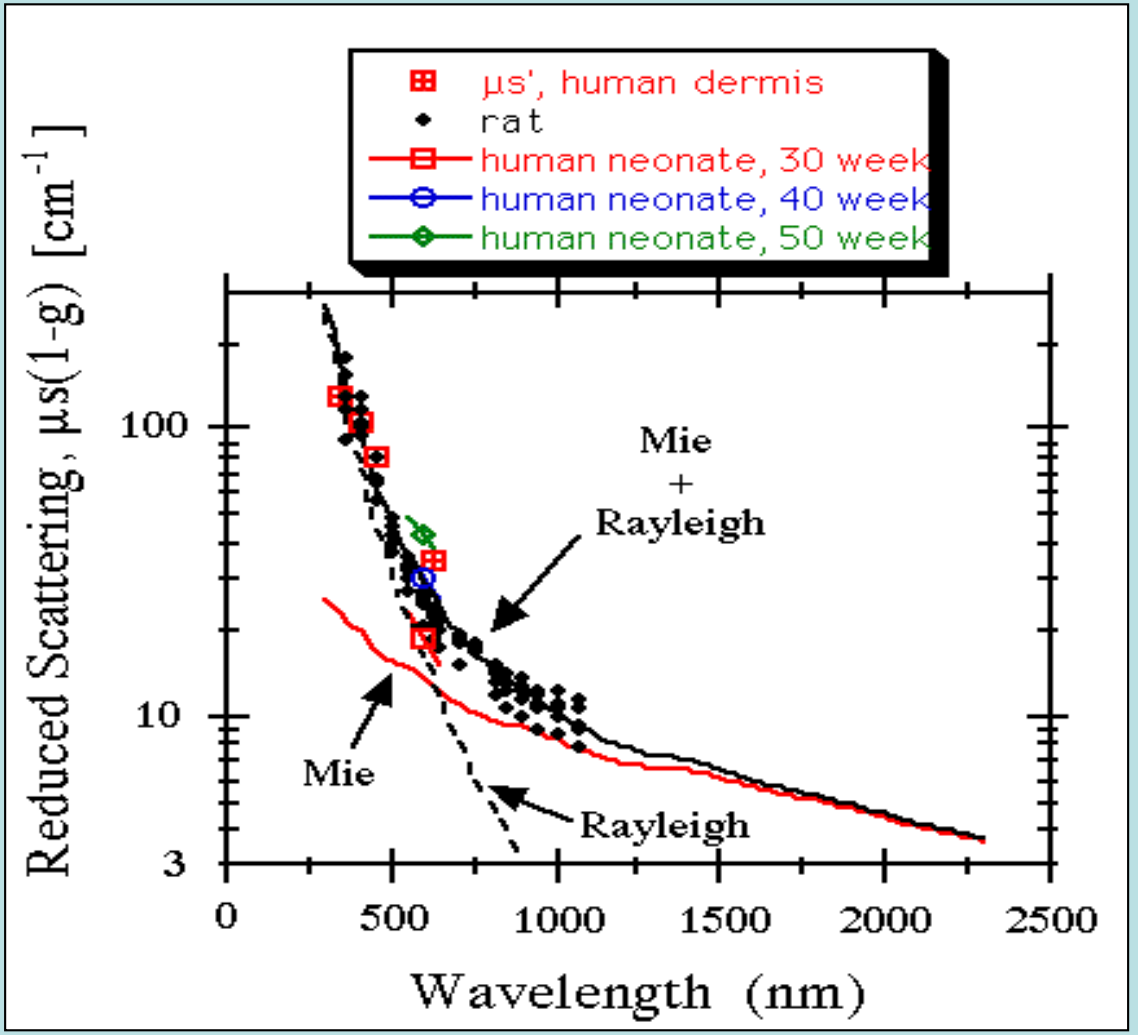
Reduced Scattering coefficient for different cylinder diameters.
Ultrastructure of collagen fibrils: ⇨ 50 nm (range 10 – 100 nm)



Biological Example: Skin/ Collagen (3)

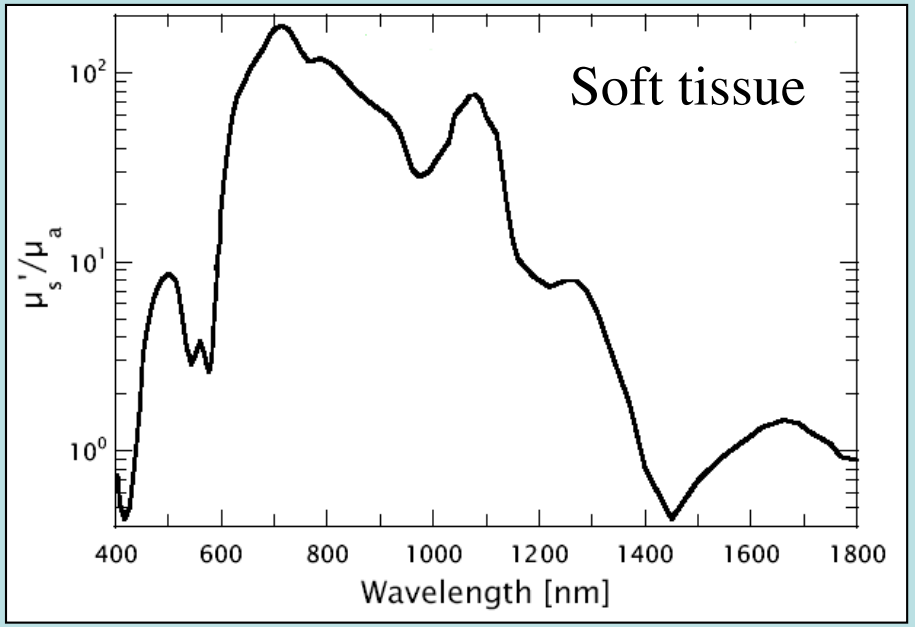
Reduced scattering coefficient: experimental (symbols) and theoretical values (lines) assuming:

(i) Rayleigh (black dotted line; n_p = 1.50, n_{med} = 1.35, fv = 0.21, 50 nm spheres)
(ii) Mie (red line; n_p = 1.46, n_{med} = 1.35, fv = 0.21, collagen fibers: 2.8 μm)
(iii) Mie & Rayleigh (black line)



Importance of the relative magnitudes of the absorption and reduced scattering coefficients

Modeling of light transport in tissues is often governed by the relative magnitudes of optical absorption and scattering



$\mu_a >> \mu_s'$: Lambert-Beer Law ($\lambda \leq 300$ nm; $\lambda \geq 2000$ nm)

$\mu_s' >> \mu_a$: Diffusion Approximation (600 nm ~ 1000 nm)

$\mu_s' \sim \mu_a$: Monte Carlo simulations
(300 nm ~ 600 nm; 1000 nm ~ 2000 nm)